# DEGRADATION OF CELLOOLIGOSACCHARIDES IN OXIDATIVE MEDIUM AND ALKALINE MEDIUM: HPLC, FTIR, AND GC-MS ANALYSES

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The chemical degradations of highly-purified cellotriose, cellotetraose, and cellopentaose in $H_2O_2$ and NaOH media were studied, respectively. The degradation products were analyzed by HPLC, FTIR, and GC-MS techniques. The results show that for the three oligosaccharides the main oxidative degradation products are 2, 3-dihydroxy-butanedioic acid, 2-keto-gluconic acid, glucopyranose, D-glucose, D-gluconic acid, and cellooligosaccharides with lower DP. A small amount of arabinose is formed during the oxidation of cellotriose. The main alkaline degradation products for the three oligomers include 3-deoxy-isosaccharinic acid-1,4-lactone and 3-deoxy-hexonic acid-1,4-lactone. Arabinose coumpounds are found to be an accidental degradation product of cellotriose. Finally, the possible formation mechanisms are proposed, including 2,3-dihydroxy-butanedioic acid, 2-keto-gluconic acid, D-gluconic acid, arabinose, 3-deoxy-isosaccharinic acid-1,4-lactone, and 3-deoxy-hexonic acid-1,4-lactone. The radical attack from $H_2O_2$ is probably at the glycosidic linkage, resulting in the formation of a series of degradation products. Degradations of cellooligosaccharides in alkaline solution are elucidated to follow an enediol anion reaction mechanism.

*Keywords: Cellooligosaccharides; Oxidative degradation; Alkaline degradation; Degradation products; Mechanism*

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## INTRODUCTION

Cellulose is one of the main components of renewable lignincellulosic biomass. Functional cellooligosaccharides can be obtained from the hydrolysis of cellulose, followed by fractionation and purification of the resulting hydrolysate (Akpinar et al. 2004; Zhang et al. 2003; Flugge et al. 1999). Cellooligosaccharides are linear oligomers of glucopyranose moieties linked by β–1,4–glycosidic bonding, as illustrated in Fig. 1. They are non-digestible oligosaccharides (NDOs) and possess important physicochemical and physiological properties beneficial to the health of consumers, such as anti-cancer, anti-inflammatory, promoting mineral adsorption, and easing diabetes and obesity (Asvarujanon et al. 2005; Mussatto et al. 2007). Because they have the same functional groups as those of cellulose, cellooligosaccharides are also used as models to study the structures and physico-chemical properties of cellulose (Ford et al. 2005; Mosier et al. 2001).

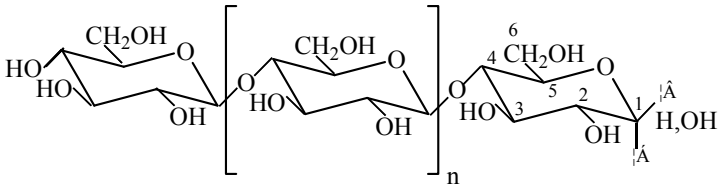

**Fig. 1.** Chemical structures of cellooligosaccharides: n=0, cellobiose; n=1, cellotriose; n=2, cellotetraose; n=3, cellopentaose; n=4, cellohexaose

Recently, there is an increasing interest in carbohydrate degradation reactions due to their role in many important fields, including food, pharmaceuticals, environmental protection, fine chemicals, and the pulp and papermaking industry (Chheda et al. 2007; Yang and Montgomery. 2007; Potgieter et al. 2005). Until now, because purified cellobiose is relatively easier to obtain, many studies related to the chemical degradation of cellobiose have been carried out. The major pathways to achieve chemical degradation of cellobiose are oxidative degradation, alkaline degradation, acid hydrolysis, and hydrothermal degradation (Roldàa et al. 2002; Moe et al. 2002; Calle et al. 1992; Berg et al. 1995; Mosier et al. 2002; Luijkx et al. 1995). 4-O-β-D-Glucopyranosyl-D-gluconic acid (cellobionic acid) is formed when cellobiose is treated by ozone (Moe et al. 2002). The degradation study of cellobiose in methyl sulfoxide-tetrabutylammonium hydroxide demonstrates that the generation of radicals and the types of radical formed are dependent on the anomeric configuration of non-reducing moieties and on the position of the linkages and deoxy group (Calle et al. 1992). However, there are very few studies on the chemical degradation of cellooligosaccharides with degree of polymerization (DP) higher than two (Sartori et al. 2003). Therefore, studies about chemical degradation are expected to have potential utilization in many fields.

At present, hydrogen peroxide and aliline usually are used in pulp and papermaking processes and have a great influence on the degradation of cellulose. In the present work, highly purified cellotriose, cellotetraose, and cellopentaose were used as models to study the effects on cellulose of hydrogen peroxide ($H_2O_2$) and NaOH. High performance liquid chromatography (HPLC), Fourier transform infrared spectrometry (FTIR), and gas chromatography-mass spectrometry (GC-MS) were applied to analyze and indentify the degradation products. Furthermore, the formation mechanisms of some products are also discussed.

## EXPERIMENTAL

### Materials

Ultra-pure water (R=18.2 MΩ) was prepared by ultrafiltration with a Milli-Q water purification system (Millipore, Bedford, MA, USA). Polyacrylamide gel Bio-Gel P-2 (fine, 45-90 μm) was purchased from Bio-Rad. Trimethylchlorosilane (TMS) (>99.9%) and hexamethyldisilane (HMDS) (>98.0%) were obtained from Sigma. All other reagents were analytical grade.

## Methods

*Preparation and fractionation of water-soluble cellooligosaccharides*

Cellooligosaccharides were prepared by means of catalyzed hydrolysis of cotton cellulose using a mixture of hydrochloric acid (37 wt%) and formic acid (88 wt%) (v:v, 4:96). The fractionation and purification of water soluble cellooligosaccharides was carried out on a Bio-Gel P-2 column (2.5 cm × 125 cm). The methods were the same as described recently (Peng et al. 2008). The purities examined by HPLC were 98.12% for cellotriose, 98.65% for cellotetraose, and 98.45% for cellopentaose.

*Oxidative degradation of cellooligosaccharides by peroxide hydrogen*

90 mg of cellotriose was dissolved with 4.5 ml of ultra-pure water in a 25 ml round flask. After the flask was heated in a water bath for 6 min at 60 °C under stirring, 1.5 ml of hydrogen peroxide (which was provided by Lianhe chemical company of Chendu) with a concentration of 30% was added dropwise, then the flask was sealed, and the reaction started. 1 h later another 1.5 ml of $H_2O_2$ was added, and the last 1.5 ml of $H_2O_2$ was added after 2 h. In total, 4.5 ml of oxidant was consumed. The oxidation reaction was continued for another 4 h at the same temperature under stirring. Neglecting the volume change throughout the reaction, the concentrations of oligosaccharide and $H_2O_2$ could be considered as 10 mg/ml and 4.9 mmol/ml, respectively. After the desired reaction time, the oxidation products mixture was directly freeze-dried, and a white powder was obtained. The oxidations of cellotetraose and cellopentaose were carried out under the same procedure as described as above.

*Alkaline degradation of cellooligosaccharides by NaOH*

Cellooligosaccharide samples were each dissolved in 10 ml of NaOH solution with a concentration of 4.8 mmol/ml in 25 ml round flasks. The concentrations of cellooligosaccharides were all 10 mg/ml. The flasks were sealed and incubated at 60 °C for 6 h under stirring. After the reactions were finished, the solutions were cooled down to room temperature immediately. Then 10 g of strong acid cation exchange resin AG 50W-X4/H+ pretreated was added to neutralize the excess NaOH and to liberate the organic acids from their salts. After filtration, the resin was washed five times, and the filtrate and washings were combined. The aqueous samples were concentrated under reduced pressure at 70 °C, then freeze-dried thoroughly. The powder product mixtures were obtained finally.

*HPLC analysis*

The samples dissolved in distilled water were analyzed on a Waters 486 HPLC system equipped with a differential refractometer (Model 600E) and a Sugar-Pak™ I column (6.5 × 300 mm). Before injection, samples were filtered through a 0.45 μm microporous cellulose membrane filter. Aliquots of filtered sample (10 μl) were injected to the HPLC system. The samples were eluted using degassed-deionized-distilled water as the mobile phase with a flow rate of 0.5 ml/min. The column temperature was 90 °C. The chromatograms were normalised through the overall peak area.

*FTIR analysis*

The KBr pellet technique was applied for determining the IR spectra of samples. The range 4000-400 $cm^{-1}$ was investigated. Spectra were recorded on a Thermo Nicolet spectrometer. KBr pellets containing 1% of sample were used.

*Derivatization of the degradation products*

12 mg of freeze-dried degradation products was dissolved in anhydrous pyridine (1.2 ml) in a tube. After heating to 50 °C, 0.8 ml of TMS and 1.6 ml of HMDS were added. The tube was heated for another 60 min at 50 °C after sealing, then cooled, dehydrated by anhydrous sodium sulfate, and centrifuged. The solution of the silylated compounds (2 μL) was injected directly into the GC-MS system.

*GC-MS analysis*

GC-MS analyses were performed on an Agilent 190915-433 GC-MS device, equipped with a HP-5MS column (30.0 m × 0.25 mm × 0.25 mm), carrier gas He, gas rate 1 ml/min, and an ICIS data system. Mass spectra were obtained by electron impact (EI) ionization at 70 eV.

The temperature program was as follows: (i) 150 °C, hold for 3 min; (ii) temperature rise from 150 °C to 230 °C at a rate of 12 °C /min; (iii) temperature rise from 230 °C to 290 °C at a rate of 7 °C /min; and (iv) 290 °C, hold for 10 min.

## RESULTS AND DISCUSSION

### Degradation Products and Proposed Mechanism of Oligosaccharide in Hydrogen Peroxide Solution

The HPLC chromatograms of the oxidative degradation products of cellotriose, cellotetraose, and cellopentaose are shown in Fig. 2. The oxidation products of cellotriose included glucose and cellobiose (Fig. 2a). The carbohydrate products of cellotetraose were glucose, cellobiose, and cellotriose (Fig. 2b). And the resultants of cellopentaose included a small quantity of glucose, cellobiose, and cellotriose (Fig. 2c). The results demonstrate that in the case of cellooligosaccharides the attack of the radical produced from $H_2O_2$ is probably at the glycosidic linkage, resulting in the formation of glucose and cellooligosaccharides with lower DP, and the resultants were further degraded (Sarbu et al. 2003). If the radical attack were to occur only in the pyranosic ring, then the chain length would remain unaltered with negligible changes of the molecular mass, and there would be no glucose and new cellooligosaccharides produced (Sarbu et al. 2003). Excepting glucose and cellooligosaccharides with lower DP, there are several new products, for example, A, B, and C as seen in Fig. 2. These unknown products may be polyhydroxy acid, aldehyde, or ketone. Therefore, other techniques such as FTIR and GC-MS would be necessary to determine the unknown products.

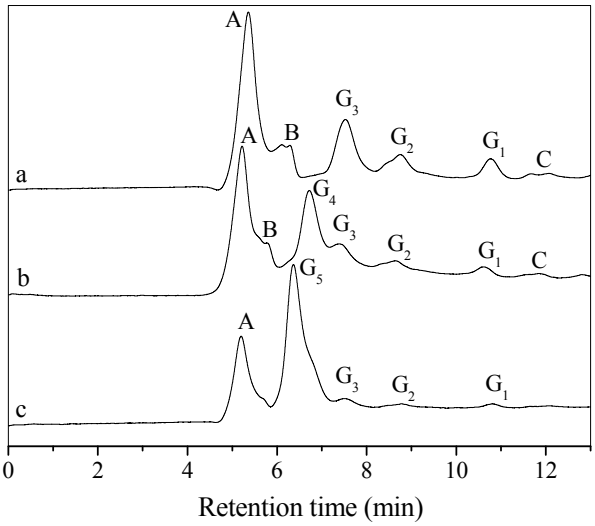


**Fig. 2.** HPLC chromatograms of the oxidative degradation products of (a) cellotriose, (b) cellotetraose and (c) cellopentaose oxided by $H_2O_2$: $G_1$, glucose; $G_2$, cellobiose; $G_3$, cellotriose; $G_4$, cellotetraose; $G_5$, cellopentaose

The FTIR spectra of the degradation products and pure cellooligosaccharides are shown in Fig. 3. The FTIR spectra of cellooligosaccharides were very similar to that of cotton cellulose. The prominent band at around 3132-3400 $cm^{-1}$ represents the hydroxyl stretching vibrations and hydrogen bonding of cellooligosaccharides and their degradation products. The C–H stretching vibrations of $–CH_2–$ give a signal at 2930 $cm^{-1}$ (Sun et al. 2004). The absorption at 1640 $cm^{-1}$ is principally associated with absorbed water. The band attributed to $–CH_2–$ bending vibration is observed at 1401 $cm^{-1}$ (Sun et al. 2004). The absorption at 1150 $cm^{-1}$ corresponds to the C–O–C stretching vibration of pyranoid ring ether (Sun et al. 2004). The prominent band at 1030 $cm^{-1}$ is attributed to the C–OH bending vibration (Sekkal et al. 1995). The peak at about 1724-1736 $cm^{-1}$ is due to the stretching vibration of carbonyl, and the small absorption at 1206 $cm^{-1}$ is attributed to the CO–O stretching vibration (Sun et al. 2004).

Compared with the FTIR spectra of pure cellotriose (g), cellotetraose (h), and cellopentaose (i), new peaks at 1724-1736 $cm^{-1}$ and 1206 $cm^{-1}$ attributable to carboxylic acid or carboxylic acid ester appear in the FTIR spectra of the degradation products mixture of cellooligosaccharides ($G_3$, $G_4$ and $G_5$,). These results demonstrate that carboxylic acid was produced during the oxidative degradation of cellooligosaccharides, and that carboxylic acid or carboxylic acid ester was produced during the alkaline degradation. The peak representing the stretching vibration of $–CH_2–$ at 2930 $cm^{-1}$ is weakened after oligosaccharides have been treated by $H_2O_2$. The peak intensity of the C–OH bending vibration at 1030 $cm^{-1}$ of the degradation products ($G_3$, $G_4$ and $G_5$,) decreases with the increase of DP of substrate cellooligosaccharide. At the same time, the peaks at 2930 $cm^{-1}$ and 1030 $cm^{-1}$ are characteristic FTIR adsorptions of carbohydrate. The results suggest that the kinds and contents of carbohydrate compounds have been greatly changed after being degraded by 4.9 mmol/ml of $H_2O_2$ at 60 °C for 6 h.

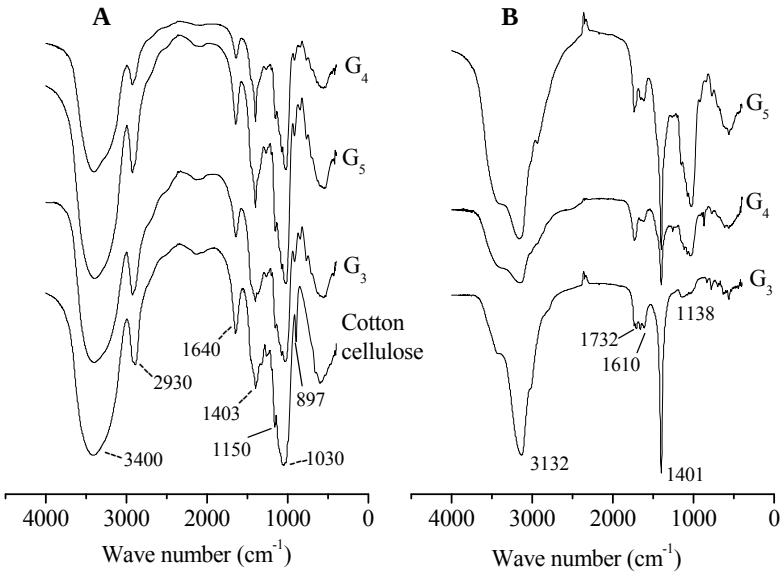


**Fig. 3.** FTIR spectra of cellooligosaccharides (A) and their degradation products (B) by $H_2O_2$. (A): $G_3$, cellotriose; $G_4$, cellotetraose; $G_5$, cellopentaose. (B): $G_3$, degradation products of cellotriose; $G_4$, degradation products of cellotetraose; $G_5$, degradation products of cellopentaose.

The GC-MS spectra of the oxidative degradation products as their trimethylsilyl derivatives are presented in Figs. 4 through 6. The GC-MS analysis results are given in Table 1. During the lyophilization process, probable products such as oxaldehyde and formic acid may be removed away because of their volatility (Isbell and Frush 1987; Manini et al. 2006). Therefore, volatile products were not determined by GC-MS. GC-MS analyses show that there are five main oxidation products, including 2, 3-dihydroxy-butanedioic acid, 2-keto-gluconic acid, glucopyranose, D-glucose and D-gluconic acid. Furthermore, there is a small quantity of arabinose resulting from the oxidation of cellotriose. Arabinose was not detected when cellotetraose and cellopentaose were oxided by $H_2O_2$.

**Table 1.** GC-MS Analysis Results of the Degradation Products of Cellooligosaccharides by $H_2O_2$

| Peak number | Compounds |
|---|---|
| 1 | 2,3-Dihydroxy-butanedioic acid (TMS) |
| 2 | 2-Keto-gluconic acid (TMS) |
| 3 | Glucopyranose (TMS) |
| 4 | D-Glucose (TMS) |
| 5 | D-Gluconic acid (TMS) |
| 6 | Pyridine |
| 7 | *p*-Phthalic acid (TMS) |
| 8 | Arabinose (TMS) |

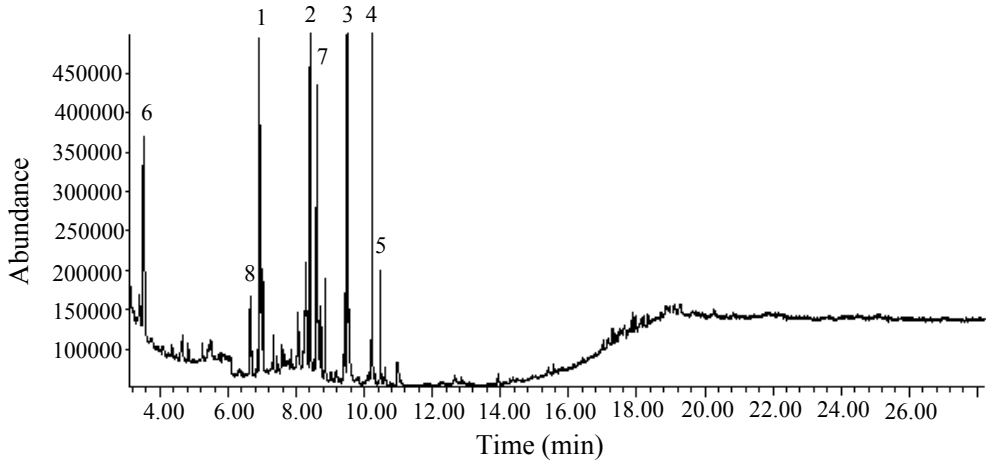


**Fig. 4.** GC–MS analysis of the degradation products of cellotriose oxided by $H_2O_2$ as their trimethylsilyl ($Me_3Si$) derivatives

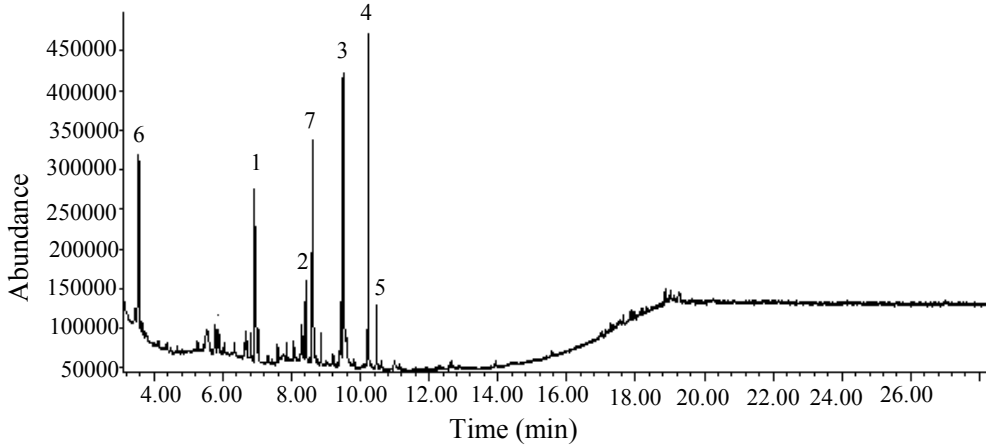


**Fig. 5.** GC-MS analysis of the degradation products of cellotetraose oxided by $H_2O_2$ as their trimethylsilyl ($Me_3Si$) derivatives

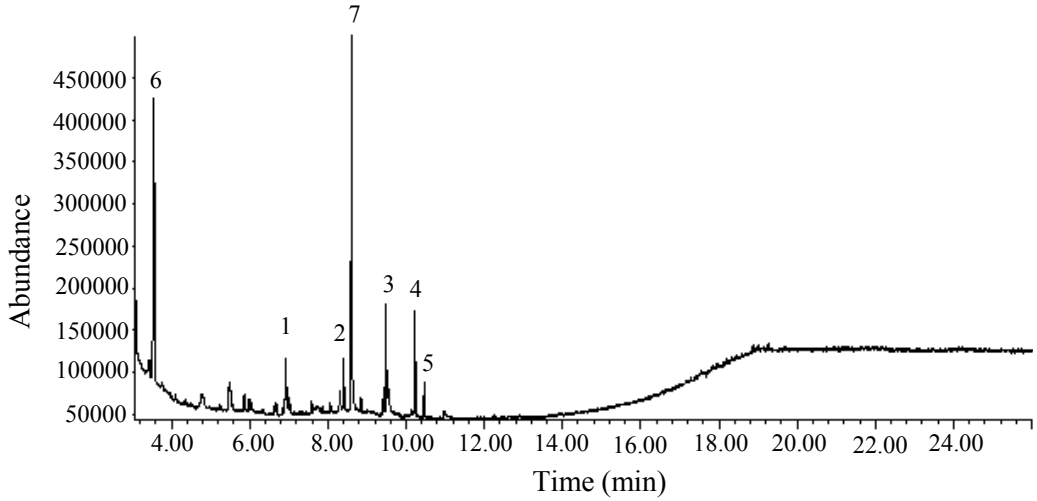


**Fig. 6.** GC–MS analysis of the degradation products of cellopentaose oxided by $H_2O_2$ as their trimethylsilyl ($Me_3Si$) derivatives

The oxidations of cellooligosaccharides by $H_2O_2$ are non-selective reactions. $H_2O_2$ is decomposed to the hydroxy radical HO•, the hydroperoxy radical HOO•, and free oxygen $O_2$. These radicals and free oxygen can act at the reducing end group, resulting in the opening of a glycosidic ring and the formation of carboxylic acid. The alcoholic hydroxyl can also be oxided to form a carbonyl group.

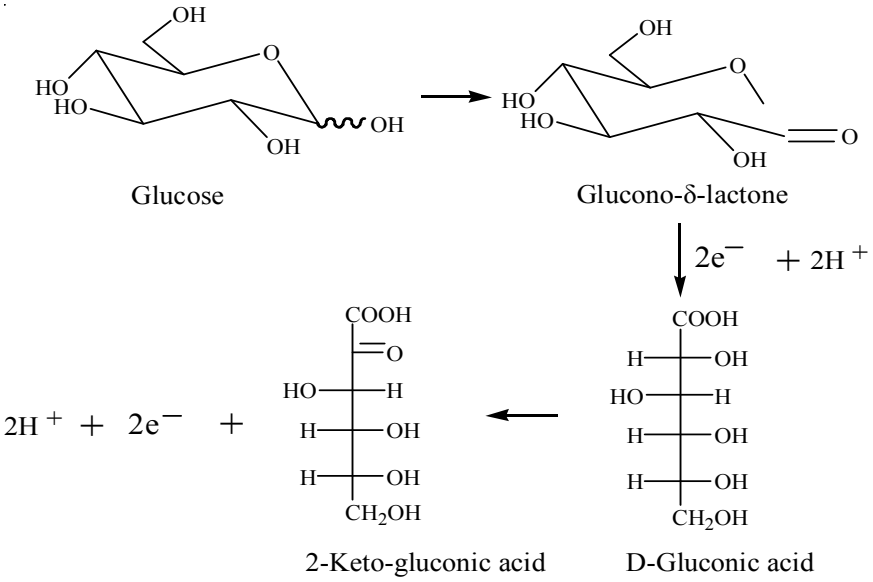


**Fig. 7.** Possible scheme of the formation of D–gluconic acid and 2–keto–gluconic acid

As the HPLC analysis results show, the glycosidic bond would break to form glucose and oligosaccharides with lower DP when cellooligosaccharides are subjected to $H_2O_2$ (Fig. 2). Glucose, which is one of the oxidative products, exists in the form of glucopyranose and ring-opening glucose, according to the GC-MS analysis results (Figs. 4 through 6). In aqueous medium, the anomeric carbon of glucose is very reactive, and the hemiacetalic function is easily to be oxided by the oxidant of $H_2O_2$, resulting in the formation of the glucono-δ-lactone, which undergoes hydrolysis, forming D-gluconic acid as the final product. At the same time, a portion of secondary alcohol hydroxyl at the C-2 position of D-gluconic acid was oxided further to give 2-keto-gluconic acid (Bamba et al. 2005). The possible scheme of the formation of D-gluconic acid and 2-keto-gluconic acid is shown in Fig. 7.

The appearance of arabinose may be due to the further oxidation of glucose produced. A possible mechanism is depicted in Fig. 8. At first, the addition reactions between the aldehydo form of glucose and $H_2O_2$ occur, resulting in the formation of a peroxidate analog. Sequently, the peroxidate analog may decompose by a free-radical mechanism, and the products are arabinose and formic acid.

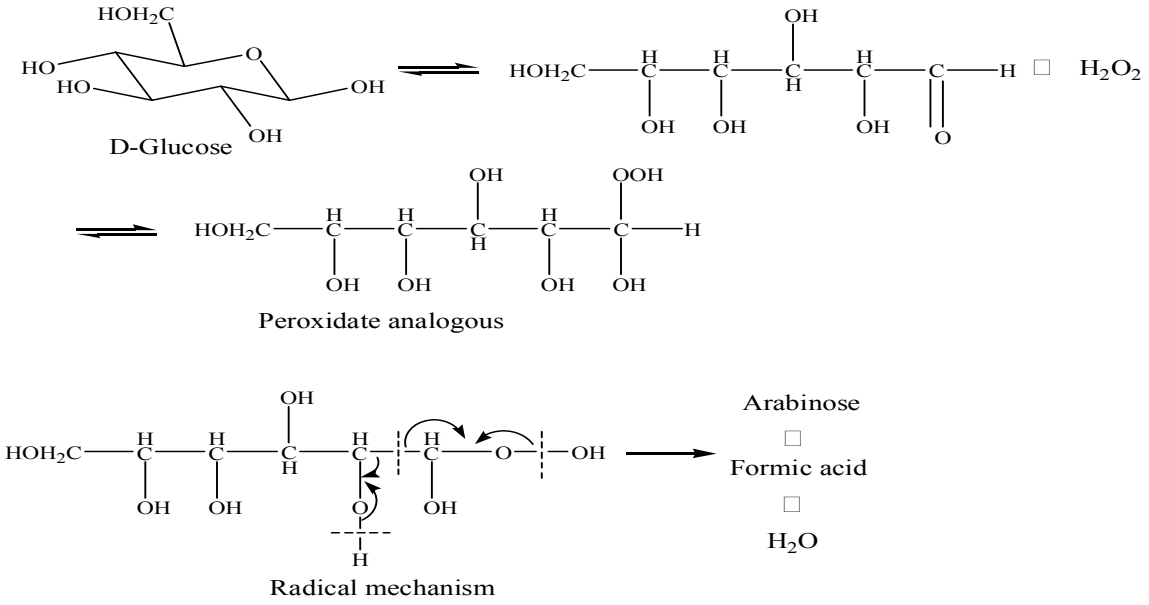


**Fig. 8.** Possible scheme of the formation of arabinose

Figure 9 shows a plausible route to form 2,3-bihydroxybutanedioic acid. A hydroxy radical abstracts H-atom of the C-2 position of glucose to produce carbon-centred radicals. Carbon-centred radicals can react with oxygen to give the corresponding peroxy radicals, which may be involved in H-atom abstraction to form the corresponding hydroperoxides. Later, the hydroperoxides transform to alkoxyl radicals. Then there is rearrangement with ring cleavage between the C-1 and the C-2, and aldehyde groups and carboxylic groups form at the C-3 and the C-2 respectively. Finally, under the action of unreacted $H_2O_2$, the ether bonds of pyranoid rings break, and the aldehyde groups and hydroxy groups at the C-1 and the C-6 are oxided to carboxylic acid, respectively. As a result, 2,3-bihydroxybutanedioic acid and oxalic acid form. Because of its instability in strongly oxidizing medium of $H_2O_2$, oxalic acid is further transformed to $H_2O$ and $CO_2$.

## Degradation Products and Proposed Mechanism of Oligosaccharide in Sodium Hydroxide Solution

The HPLC chromatograms of the alkaline degradation products of cellotriose, cellotetraose, and cellopentaose are shown in Fig. 10. The alkaline products of cellotriose included glucose and some unknown compounds (Fig. 10a). But oligosaccharides with DP lower than the reactant are absent in the degradation products of cellotetraose and cellopentaose (Fig. 10b and c). It may be that the content is too low to be detected by HPLC.

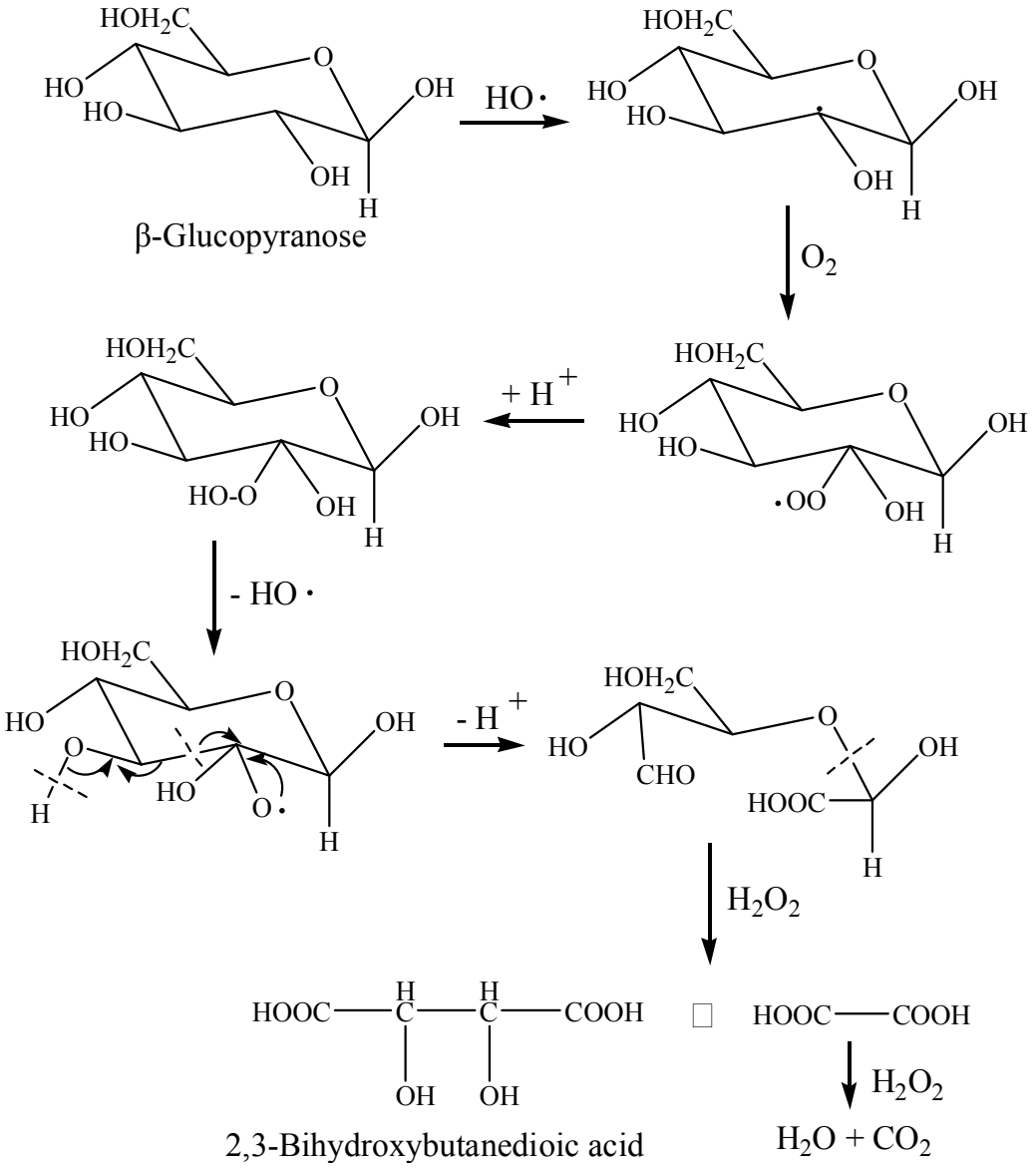


**Fig. 9.** Possible scheme of the formation of 2,3-bihydroxybutanedioic acid

The FTIR spectra of the alkaline degradation products are shown in Fig. 11. The characteristic peaks of alkaline degradation products are consistent with that of oxidation degradation products and oligosaccharides. As with oxidation degradation products, new peaks at 1724-1736 $cm^{-1}$ and 1206 $cm^{-1}$ appear in the FTIR spectra of the alkaline degradation products. The stretching vibration of —$CH_2$— at 2930 $cm^{-1}$ is also weakened after the oligosaccharides were treated with NaOH. The peak intensity of the C—OH bending vibration at 1030 $cm^{-1}$ of the degradation products decreases with the increase of DP of substrate cellooligosaccharide. The results indicate the carbonylcompound was produced during the oxidative degradation of cellooligosaccharides, but the certain compounds need further analysis.

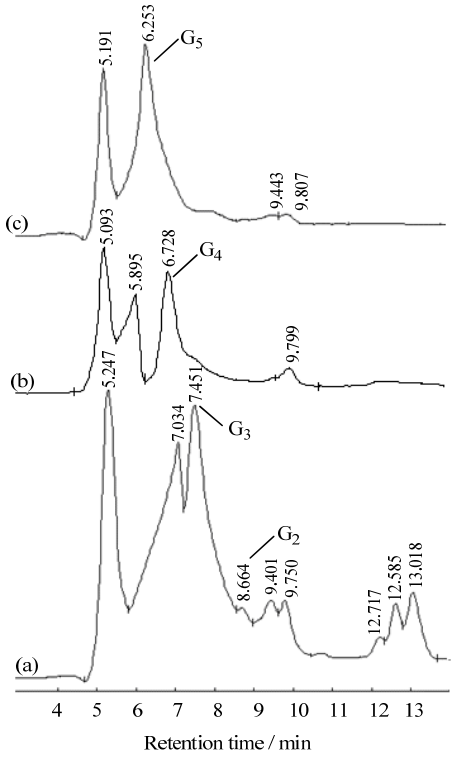


**Fig. 10.** HPLC chromatograms of the alkaline degradation products of (a) cellotriose, (b) cellotetraose, and (c) cellopentaose degraded by NaOH: $G_1$, glucose; $G_2$, cellobiose; $G_3$, cellotriose; $G_4$, cellotetraose; $G_5$, cellopentaose

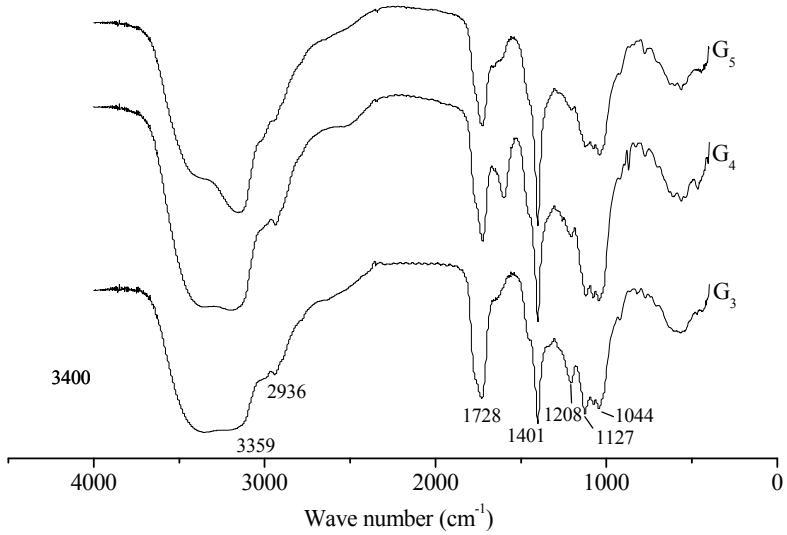


**Fig. 11.** FTIR spectra of degradation products of cellooligosaccharides in NaOH solution. $G_3$, degradation products of cellotriose; $G_4$, degradation products of cellotetraose; $G_5$, degradation products of cellopentaose

An extremely complex mixture can form when a simple monosaccharide is treated by alkali at an elevated temperature (Ericsson et al. 1972; Forsskahl et al. 1976). These products include furanoid, benzoid, and other aromatics, many of which are antioxidants (Ericsson et al. 1972; Forsskahl et al. 1976). Generally, water-soluble cellooligosaccharides are linear oligomers with DP from two to seven, which are linked by β-1,4-glycosidic bonds (Fig. 1). Therefore, the alkaline degradation products are more complex compared with the products of simple sugar treated under the same conditions. The GC-MS spectra of the degradation products as their trimethylsilyl derivatives are shown in Figs. 12 through 14. The GC-MS analysis results are given in Table 2. There are two main degradation products for the three oligosaccharides, 3-deoxy-isosaccharinic acid-1,4-lactone and 3-deoxy-hexonic acid-1,4-lactone. However, for cellotriose, arabinose, arabinopyranse, and arabinofuranose also formed during the degradation by 4.8 mmol/ml of NaOH at 60 °C for 6 h, as shown in Fig. 12 and Table 2. The unreacted cellooligosaccharides and their corresponding TMS derivatives are insoluble in pyridine; thus the residual oligomers were not detected by GC-MS for the two kinds of degradation reactions. The compound *p*-phthalic acid originated from the pyridine solvent.

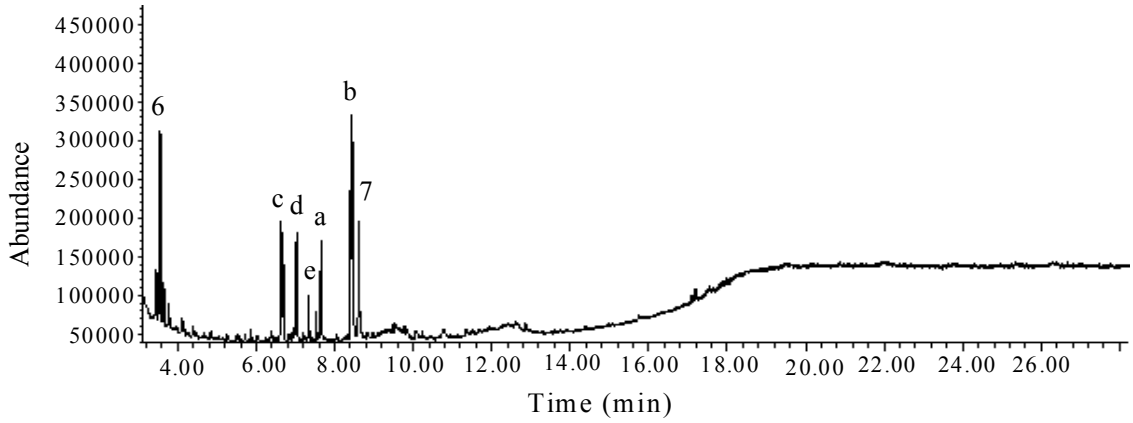


**Fig. 12.** GC–MS analysis of the degradation products of cellotriose by NaOH as their trimethylsilyl ($Me_3Si$) derivative

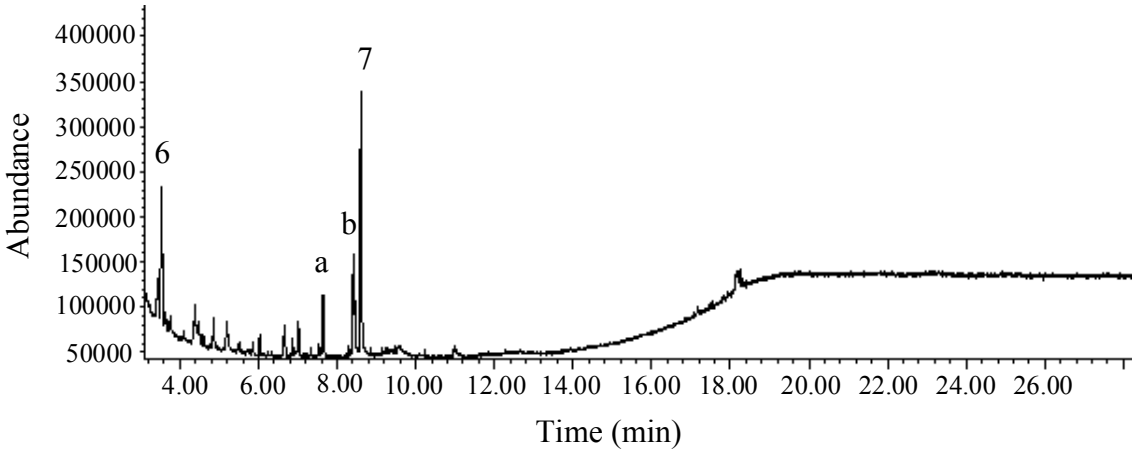


**Fig. 13.** GC–MS analysis of the degradation products of cellotetraose by NaOH as their trimethylsilyl ($Me_3Si$) derivative

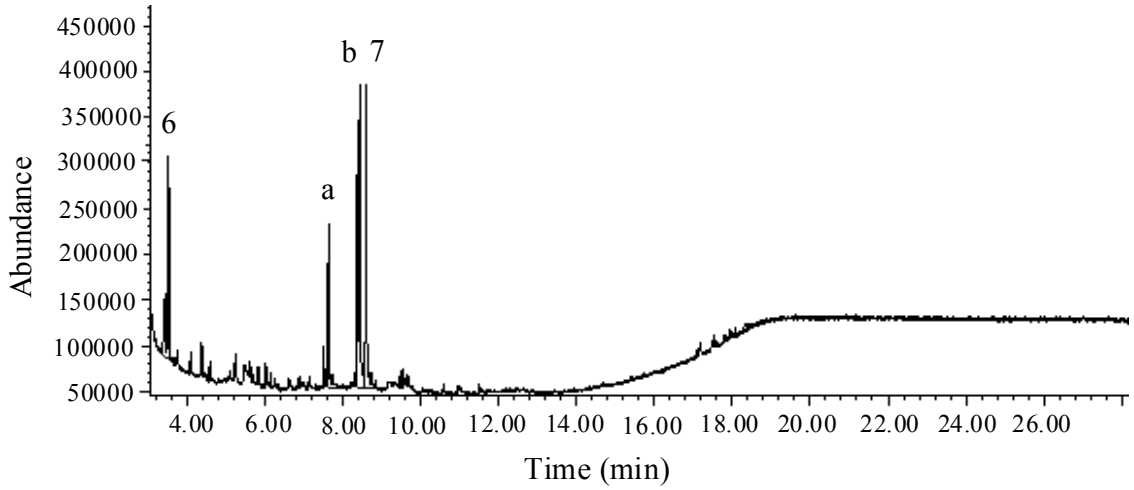


**Fig. 14.** GC–MS analysis of the degradation products of cellopentaose by NaOH as their trimethylsilyl ($Me_3Si$) derivative

**Table 2.** GC-MS Analysis Results of the Degradation Products of Cellooligosaccharides by NaOH

| Peaks | | Compounds | Content (%) |
|---|---|---|---|
| Cellotriose | a | 3-Deoxy-isosaccharinic acid-1,4-lactone (TMS) | 15.19 |
| | b | 3-Deoxy- hexonic acid-1,4-lactone (TMS) | 37.53 |
| | c | Arabinose (TMS) | 24.24 |
| | d | Arabinopyranose (TMS) | 16.14 |
| | e | Arabinofuranose (TMS) | 6.90 |
| Cellotetraose | a | 3-Deoxy-isosaccharinic acid-1,4-lactone (TMS) | 35.01 |
| | b | 3-Deoxy- hexonic acid-1,4-lactone (TMS) | 64.99 |
| Cellopentaose | a | 3-Deoxy-isosaccharinic acid-1,4-lactone (TMS) | 28.28 |
| | b | 3-Deoxy- hexonic acid-1,4-lactone (TMS) | 71.72 |
| | 6 | Pyridine | |
| | 7 | *p*-Phthalic acid (TMS) | |

Alkaline treatment of reducing sugars in aqueous solution causes isomerization, and the intermediate formation of an enediol anion species in this process has generally been accepted (Wit et al. 1979; Lai 1973). The probable mechanism of the formation of 3- deoxy-isosaccharinic acid-1, 4-lactone is shown in Fig. 15.

Firstly, the pyranoid ring at the reducing end opens to yield an aldehydo-form of cellooligosaccharide. The 1,2-enediol intermediate forms when the aldehydo-form of cellooligosaccharide is subjected to alkaline conditions. The rearrangement of the 1,2-enediol intermediate would result in the formation of 2,3-enediol intermediate. The elimination of an alkoxyl group from the β-position of the 2,3-enediol intermediate leads to the formation of an α-dicarbonyl compound (α-diketone), which is extremely unstable under alkaline conditions. Cellooligosaccharide with lower DP is formed via the hydration of the alkoxyl group. 3-deoxy-isosaccharinic acid-1, 4-lactone is formed under

the condition by the dehydration of isosaccharinic acids, which are the benzilic acid rearrangement products of α-diketone (Luijkx et al. 1995; Lloyd et al. 1995).

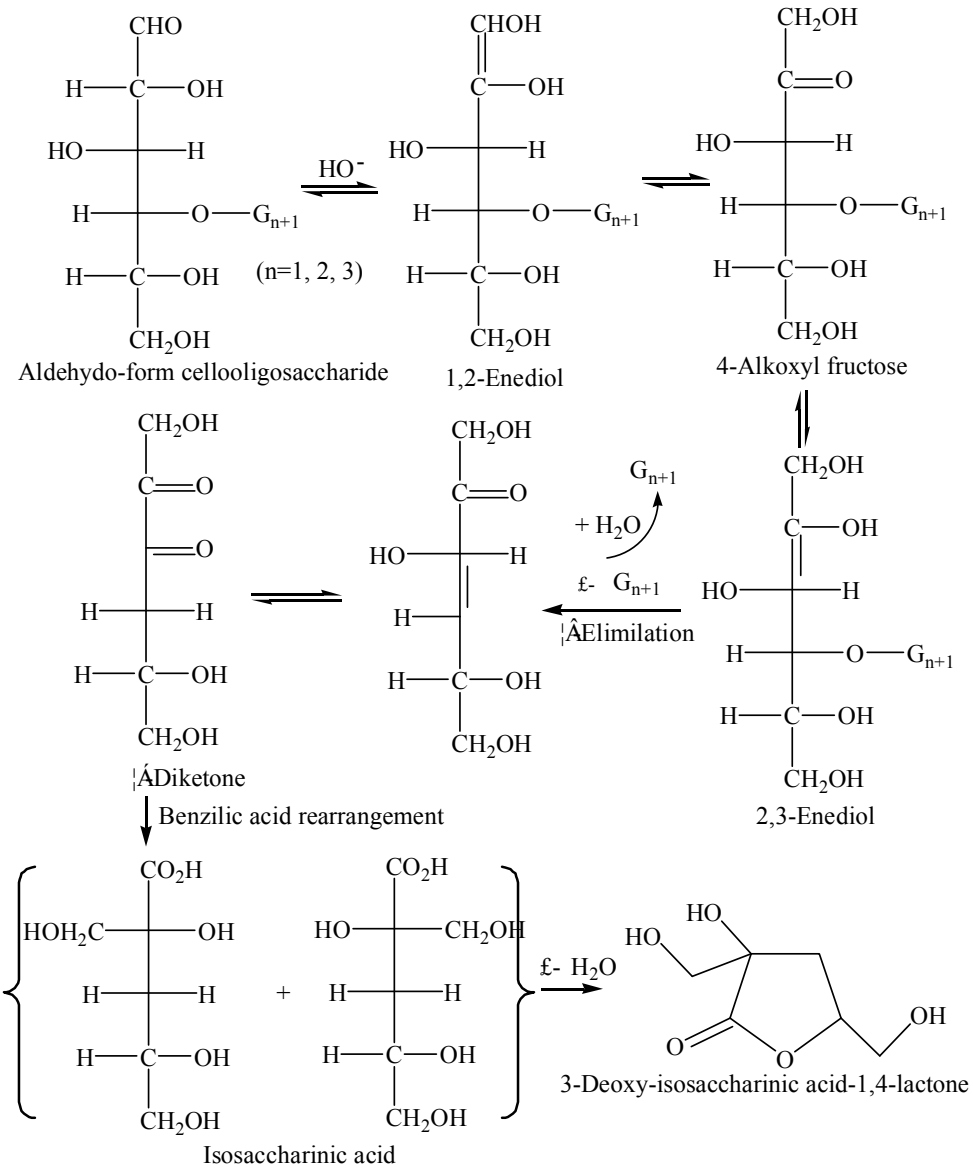


**Fig. 15.** Probable scheme of the formation of 3-deoxy-isosaccharinic acid-1,4-lactone

Figure 16 shows the probable pathway of the formation of 3-deoxy-hexonic acid-1,4-lactone. The direct β-elimination followed by rearrangement of 1,2-enediol results in ketoaldehyde, which belongs toα-dicarbonyl compound. By an intramolecular Cannizzaro reaction, 4-alkoxyl metasaccharinic acids are formed, which finally transform to 3-deoxy-hexonic acid-1,4-lactone via condensation and dehydration (Lloyd et al. 1995). At the same time, the hydration of the alkoxyl group results in cellooligosaccharide with lower DP.

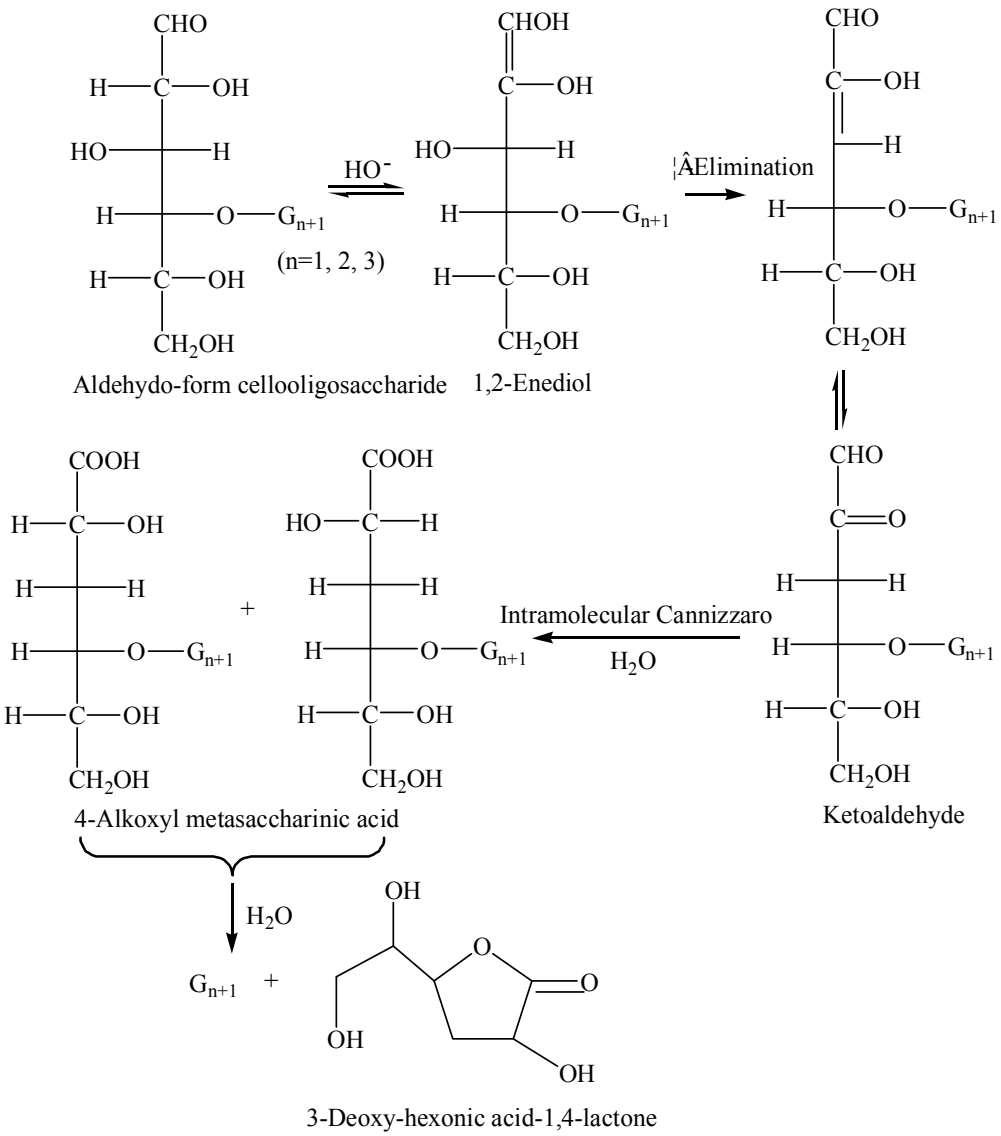


**Fig. 16.** Probable scheme of the formation of 3-deoxy-hexonic acid-1,4-lactone

## CONCLUSIONS

The oxidative degradations and the alkaline degradations of highly purified cellotriose, cellotetraose, and cellopentaose were studied. After the oxidative degradations of cellooligosaccharides by $H_2O_2$, excepting for lower DP cellooligosaccharides produced, five other main degradation products were detected, including 2,3-dihydroxy-butanedioic acid, 2-keto-gluconic acid, glucopyranose, D-glucose, and D-gluconic acid. A small quantity of arabinose was also found after the oxidation of cellotriose by $H_2O_2$. The GC-MS analyses demonstrate identical fragmentation patterns of the three main acid products, including 2,3-dihydroxy-butanedioic acid, 2-keto-gluconic acid and D-gluconic acid. The results clearly show that the radical attack

probably occurs at the glycosidic linkage, resulting in the formation of cellooligosaccharides with lower DP and glucose; simultaneously a reaction occurs leading to further degradation.

For the alkaline degradations of the three oligosaccharides, there are two main degradation products, namely 3-deoxy-isosaccharinic acid-1,4-lactone and 3-deoxy-hexonic acid-1,4-lactone. The degradation products of cellotriose, including arabinose, arabinopyranse, and arabinofuranose, were also formed. Probable mechanisms of the formation of 3-deoxy-isosaccharinic acid-1,4-lactone and 3-deoxy-hexonic acid-1,4-lactone were proposed, based on an enediol intermediate mechanism processing enolization, β-elimination, and rearrangement reactions.

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