

Full Length Research Paper

L-Arabinose and oligosaccharides production from sugar beet pulp by xylanase and acid hydrolysis

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Xylanase and sulfuric acid were used to hydrolyze sugar beet pulp for the production of L-arabinose and oligosaccharides. Xylanase was obtained by the solid state fermentation of *Thermomyces lanuginosus* DSM10635. Xylanase or dilute sulfuric acid hydrolysis was adopted to hydrolyze sugar beet pulp. The hydrolysates were quantitatively identified by high-performance liquid chromatography (HPLC). The content of arabinose was 48.4% in the total monosaccharide of sulfuric acid hydrolysate, whereas, more oligosaccharides were obtained from sugar beet pulp hydrolysate of xylanase. This study has established a new way for arabinose production, as well a potential method for oligosaccharides production.

Key words: Xylanase, hydrolysis, sugar beet pulp, L-arabinose, HPLC.

INTRODUCTION

Hemicellulose is the second most abundant plant polysaccharide and an attractive energy feedstock for the production of bioethanol. Xylan is the major hemicellulose component in nature. The sugars in xylan are primarily pentoses (D-xylose and L-arabinose) and harbor as minor constituents' hexoses (D-galactose, D-glucose and D-mannose) as well as uronic, acetic and cinnamic acids (Collins et al., 2005; Squina et al., 2009). Complete depolymerization of xylan is accomplished by the synergistic action of endo-xylanases and xylosidases along with arabinofuranosidases, ferulic acid esterases, uronidases and other enzymes, which respectively act on the xylan backbone, side chains and decorating units, producing fermentable xylo-ligomers and monomers (Remond et al., 2010).

L-arabinose is an important intermediate raw material in the pharmaceutical and fine organic synthesis industries. L-arabinose unit is a precursor in many bio-active substances structures, such as antibiotics. In food health industry, arabinose shows a blocking effect on the

metabolic conversion of sucrose (Xiong et al., 2005). Currently, arabinose is mainly produced by hydrolyzing the scarce and expensive Arabic gum which was very expensive. As the arabinose demands rapidly increased and the resources of Arabic gum are limited, the price of arabinose will rise higher and higher. To develop a cheaper arabinose production process became an urgent task.

Oligosaccharides are small polymer with 2 to 10 monosaccharides joint to form straight or branched-chain by means of the glucoside bonds (Liab et al., 2000; Teramoto et al., 2008). Among kinds of oligosaccharides, Xylo-, Mannan-, Fructo-, and α -Gluco-oligosaccharides were mainly fed to animal as feed additive. There are many good physical and chemical properties of oligosaccharides, such as low heat, stability, security, non-toxic and no residue, etc. Oligosaccharides improve immunity, feeding efficiency and the quality of livestock products (Uma et al., 1999). At present, oligosaccharides is extracted from corn, soybean and yeast strain directly and it is also obtained by the reactions of artificial polysaccharide decomposition, monosaccharide binding and glycosyl transferase transfer (Crich and Vinogradova, 2007).

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Due to the enrichment of protein in the feedstock, the Maillard reaction happened; a large number of free amino acids are generated during the acidic hydrolysis. At that hydrolysis process, the produced monosaccharide reacted with those free amino acids, which resulted in the color reaction and a large number of sugars being consumed (Ames, 1998; Zhang et al., 2009). There are many advantages of xylanase hydrolysis, such as mild conditions, high efficiency, etc (Gomes et al., 1993; Oosterveld et al., 2000).

The goal of this study was to obtain the different productions by two different methods. L-arabinose as the production of enzyme-acid hydrolysis was 48.4% in the monosaccharides of total hydrolysate, whereas, more oligosaccharides were obtained from sugar beet pulp hydrolysate of xylanase. This study has established a new way to produce L-arabinose and oligosaccharides.

MATERIALS AND METHODS

Cultivation conditions

The composition of the liquid culture medium was 2 g/l potato, 0.2 g/l glucose and deionized water. The inoculate activated *Thermomyces lanuginosus* DSM10635 strain into seed liquid culture medium which was maintained at 55°C and 180 rpm agitation in a shake flask (Xiong et al., 2004). After 72 h growth, the medium was as the inoculum for solid cultivation by about 10% (v/w). The cultivation parameters were as follow; temperature 55°C, pH 6.5 and cultivation time of 9 days. Solid medium was made up by the following methods; corncob (from Shan Dong province of China) was crushed into powder and 200 g corncob powder was put into 1000 ml large beaker, with the addition of yeast extract (10 g/l), phosphate buffer (pH 6.5, 50 mM) and 3% (NH₄)₂SO₄ with the proportion of 1:3.8 (200 g corncob: 760 ml solution) and stirring evenly. The mixture was divided into six conical flasks of 1000 ml (the medium of each flask was about 160 g) and sterilized for 20 min at 121°C.

After the culture, crude enzyme solution was extracted by adding citric acid - phosphate buffer (pH 6.5, 50 mM). The supernatant was collected by centrifugation at 5000 rpm for 30 min. Then ammonium sulfate powder was added to 70% saturated and the sediment was collected by centrifugation 5000rpm for 30min at room temperature. The sediment was dissolved by 20 ml citric acid - phosphate (50 mM) buffer (pH 6.5) , and then finally kept at 4°C until use.

Preparation of plant hydrolysates

Sugar beet pulp (from Hei LongJiang province of China) was pre-treated by crude xylanase solution (500 IU/ml) at 60°C for 24 h. After the filtration, the filtered solution was then hydrolyzed by 2% sulfuric acid at 100°C for 1 h. After hydrolysis, the solution was filtered with Avanti J-E (Beckman Coulter). Then, the filtrate was boiled and the excessive dilute sulphuric acid was neutralized by calcium carbonate (CaCO₃). The precipitate was removed by centrifugal machine and stored at 4°C refrigerator for the next HPLC analysis (Xiong et al., 2005).

The direct xylanase hydrolysate sample was obtained similarly as earlier methods, but without any acid-treatment. The sugar beet pulp was hydrolyzed by the xylanase solution (500 IU/ml) at 60°C for 24 h. After hydrolysis, the solution was concentrated by vacuum evaporation at 50°C to only 20% (w/w) dry matter and re-filtered.

The hydrolysate was analyzed by HPLC.

Xylanase assays

The xylanase activity was analyzed through the 3,5-dinitrosalicylic acid (DNS) method (Bailey et al., 1992; Xiong et al., 2004). The amount of reducing sugars released during 10 min in a reaction mixture containing 0.5% xylan and 50 mM citric acid- phosphate buffer (pH 6.5) at 50°C was assayed. Then, 3 ml DNS solution was added, mixed fully, and made to boil for 5 min. Finally, the solution was made to cool to room temperature and the adsorption value was determined at a wavelength of 540 nm. The adsorption value was incorporated into the standard curve equation, and then the xylose content was obtained. An international standard unit of enzyme activity is defined as, under the conditions of 60°C and pH 6.5 is the amount of enzyme required for hydrolyzing xylan to produce 1µmol reducing sugar per minute.

Sugar assays

The different concentrations of sugar were analyzed by high-performance liquid chromatography (HPLC) (Shimadzu LC-20AT). The refractive index detector (RID-10A) system was used. The components were separated in Shodex ks – 801 at 80°C with distilled water as the mobile phase, the elution rate was 1 ml/min and the injection volume of the sample was 20 µl. The model mixtures were prepared by pure sugar based on the HPLC guideline.

RESULTS

Determination of enzyme activity

Thermomyces lanuginosus DSM10635 was cultivated under the optimal culture conditions of 60°C and pH 6.5, for 9 days. The harvested activity of xylanase was 13305 IU/ml which was diluted to the correct xylanase concentration for the enzymatic hydrolysis as in methods.

Standard HPLC curves of glucose, xylose and arabinose

Because of the molecular sieve and ion-exchange material effect of the padding in column Shodex ks-801, high molecular weight material passed the column earlier. Glucose remained at 7.526 min in the column, xylose was at 8.054 min and arabinose was at 8.627 min (Figure 1). Peak time error of sugar with different operation batches was less than 0.1 min. There were stable peak time and obvious separation for glucose, xylose and arabinose. Xylose and arabinose are isomers with the same molecular weight, but were different in ionic strength. So they were separated effectively by Shodex ks - 801.

Analysis of sugar beet pulp hydrolysate

Xylanase hydrolyzed hemicellulose into oligosaccharide and the hydrolysis fluid was further hydrolyzed by dilute

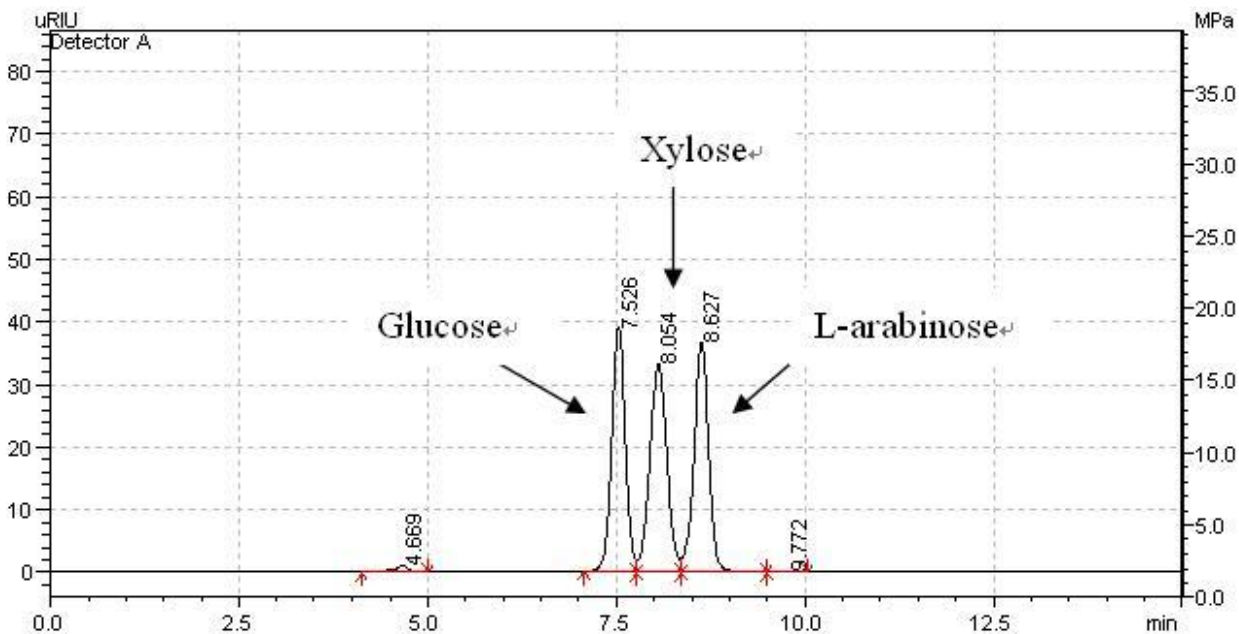


Figure 1. Standard curves of glucose, xylose and L-arabinose.

Table 1. The contents of each single sugar in beet pulp hydrolyzate.

Parameter	Glucose	D-Xylose	L-arabinose
Sugar contents (mg/ml)	2.7	2.2	4.6
Percentage (%)	28.4	23.2	48.4

acids (2% H₂SO₄, v/v). By HPLC analysis, a few sugars such as glucose, xylose and arabinose were found in the sugar beet pulp hydrolysate, but arabinose was significantly higher than the levels of glucose and xylose. The purity of the three kinds of sugar content, respectively, was 2.7, 2.2 and 4.6 for glucose, xylose and arabinose (Table 1). The dry weight of arabinose accounted for 2.76% (w/w) of the sugar beet residue.

There was a large peak with the column residual time (4.888 min) (Figure 2). Based on the separation principle of column Shodex ks-801, the material which was greater than 1000 Da molecular weight appeared on the peak at this time. This large peak did not appear at the mixed sugar standard curves (Figure 1), but appeared after the acid hydrolyzed the starch and xylan (Figures 3 and 4). So, it could be that the peak was polysaccharide. The differences of the polysaccharide peak time (4.888, 5.036 and 5.046 min in Figures 2, 3 and 4, respectively) may have been caused by the various molecular weight of the polysaccharides.

In Figure 5, there was a peak at 6.240 min between polysaccharide and glucose. Because of the separation principle of column Shodex ks-801, this peak at 6.240 min corresponded to oligosaccharides, whose molecular weight was less than the polysaccharide (peak at 4.772

min) but larger than the monosaccharides (peaks retained time larger than 7.539 min).

DISCUSSION

Agricultural wastes (like sugar beet pulp, corn cob, etc.) comprehensive utilization is currently a hot topic of scientific research. There are a large number of agricultural wastes which have not been fully exploited so, most of the resources are constantly being wasted. Xylanase hydrolysis of agricultural waste which turns waste into treasure will make the full use of waste resources and protects the environment protected.

Strong acid and strong alkali are mainly used in the traditional method of hemicellulose hydrolysis in high temperature condition. It is costly and harmful for the environments. At present, arabinose and xylose as functional or medical precursors are widely used in food and pharmaceutical industries. With the growing social demand of the mentioned matters, their prices will be higher. In this work, through enzyme-dilute acid or sole enzyme hydrolysis, arabinose and xylose were obtained from the cheap sugar beet pulp. Especially, the content of arabinose in the total sugar was more than 45%. The

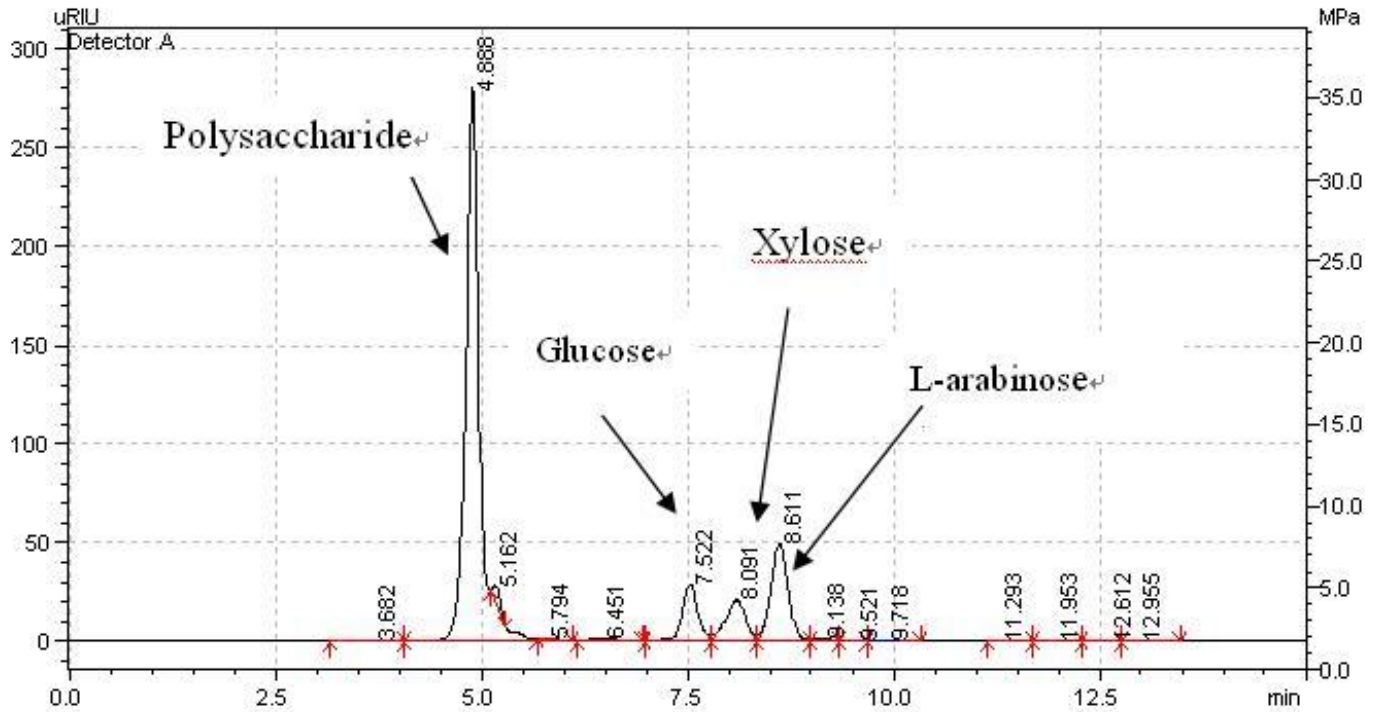


Figure 2. HPLC chromatography of xylanase-dilute acid hydrolysate.

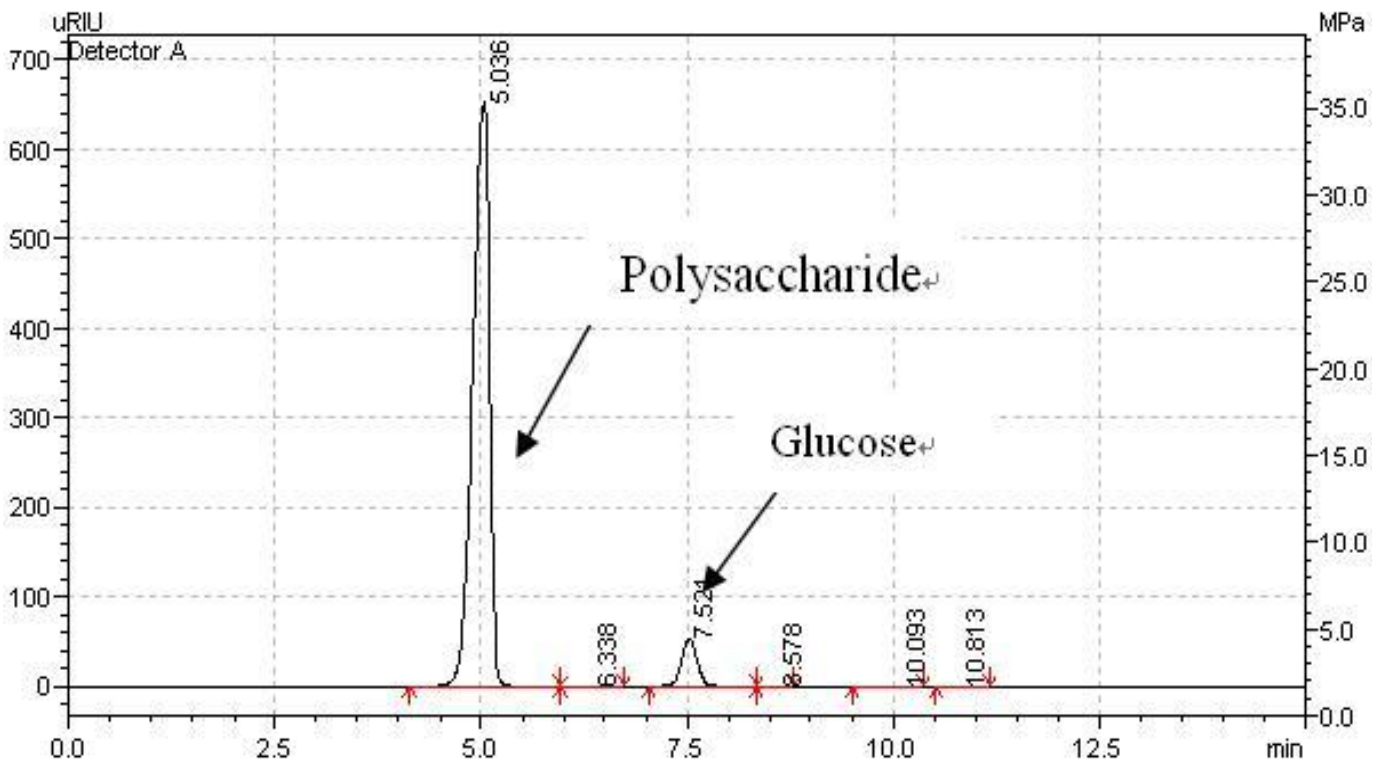


Figure 3. HPLC chromatography of acid hydrolysate of starch.

methods in this study showed ways to produce rare sugar from the cheap raw materials.

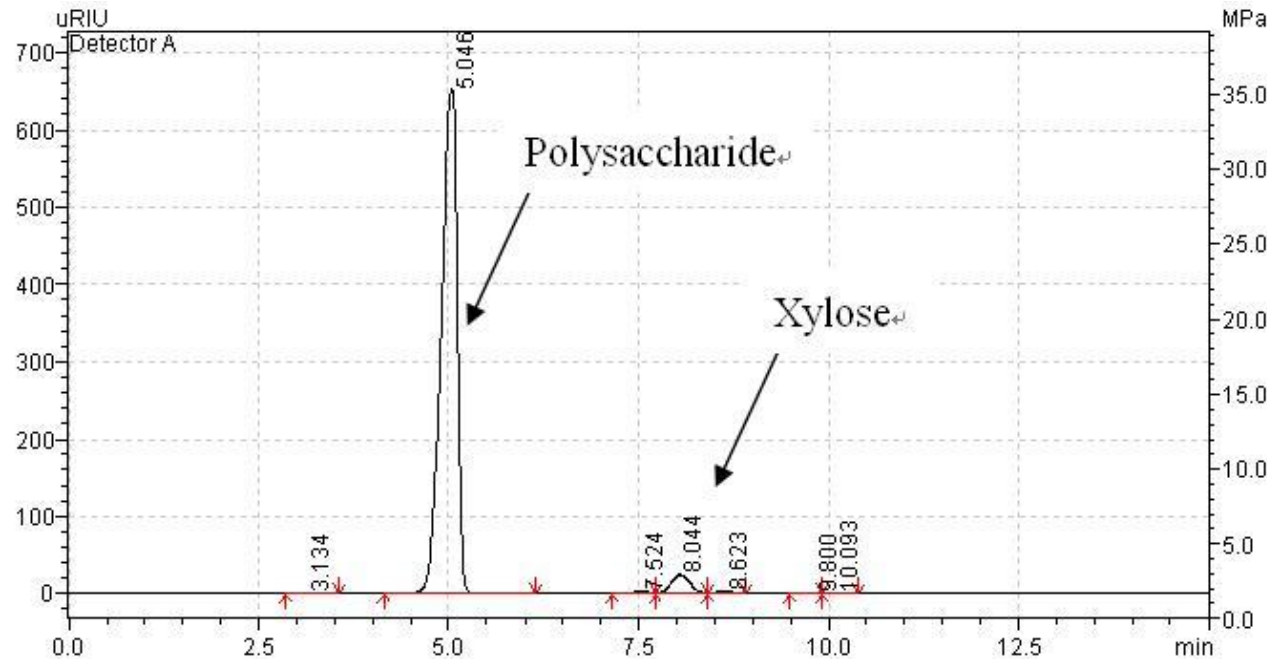


Figure 4. HPLC chromatography of acid hydrolysate of xylan.

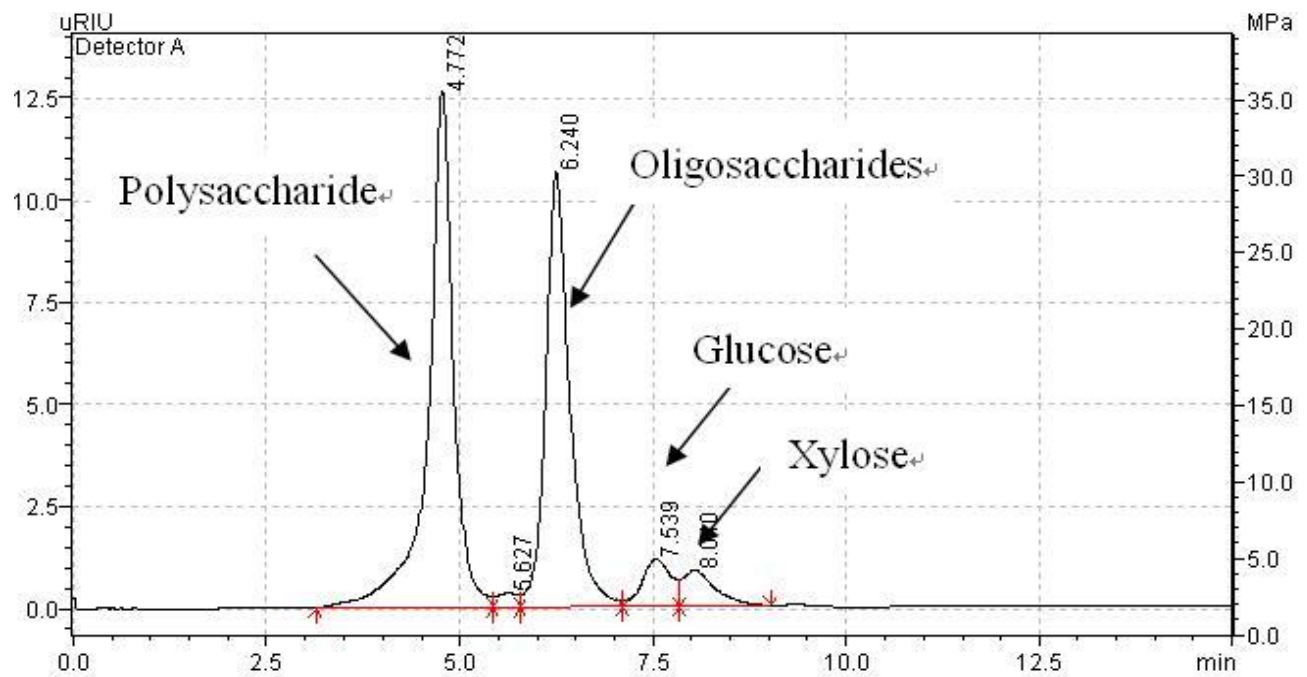


Figure 5. HPLC chromatography of hydrolysate by xylanase (500 IU/ml).

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