Note

Production of 1-L-Arabinose and Xylose from Corn Hull and Bagasse*  
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Abstract: Production of 1-L-arabinose and xylose from arabinoxylan in corn hull and bagasse was investigated using dilute hydrochloric acid treatment at 125 or 130°C. In the corn hull arabinoxylan, which has a high 1-L-arabinose content (Ara/Xyl = 0.590), 1-L-arabinose was more preferentially released than xylose as a result of less than 5 min treatment with 0.2 M HCl at 125°C. The yield from arabinoxylan was 73% after 5 min treatment, but high 1-L-arabinose ratio of 60% was obtained in the released xylose and 1-L-arabinose mixture. After 20 min treatment, the amounts of 1-L-arabinose and xylose were increased to 162 and 290 mg/g corn hull, and their yields from the corn hull arabinoxylan were 89 and 99%, respectively. Furthermore, during enzymatic hydrolysis using cellulase, glucose was significantly released from the HCl-treated corn hull. On the other hand, in the bagasse arabinoxylan which has a low 1-L-arabinose content (Ara/Xyl = 0.073), the 1-L-arabinose yield from arabinoxylan was 99%; however, the xylose yield was smaller (63%) and glucose release was not enhanced with enzymatic hydrolysis because of higher lignin content.

Key words: 1-L-arabinose, xylose, arabinoxylan, hydrochloric acid, corn hull, bagasse

Agricultural and food wastes are abundant biomass resources, which can be used for the production of fuel ethanol and food materials. Corn hull (hull of corn kernel) and bagasse are one such renewable resource, wasted in great quantities in industrial starch and sucrose production, respectively. The major component of corn hull, arabinoxylan, has a high 1-L-arabinose content—1-L-arabinose/xylose $= 0.590^{\dagger}$ and its functional potential is thus highly attractive. 1-L-Arabinose inhibits intestinal sucrase activity and suppresses increasing plasma glucose, and is therefore expected to be important for diabetic therapy. Arabinoxylan of bagasse has higher xylose content (Ara/Xyl = 0.073). Xylose, another major sugar constituent, is also potentially useful, for example, in the production of xylitol, a five-carbon sugar alcohol with an acidogenic function that is used as a food sweetener. Although xylitol is synthesized by reduction of xylose through chemical reactions, fermentation by organisms also effectively utilizes the hydrolysate mixture recovered from cellulosic materials. Furthermore, 1-L-arabinose and xylose can also be used as raw materials for fuel ethanol production as well as glucose. Previously, recombinant bacteria, in which pyruvate decarboxylase and alcohol dehydrogenase genes from *Zymonas mobilis* were integrated, were used to efficiently produce ethanol from xylose and 1-L-arabinose.5,6

Dilute-acid treatment at high temperature is studied as a pretreatment which removes the lignin barrier and improves the enzymatic hydrolysis for cellulose in wood.8,11

The treatment for 1-L-arabinose and xylose production from arabinoxylan in agricultural and food wastes, however, has not been studied in detail. In this study, using heat-treatment with water alone or dilute-acid, we discussed 1-L-arabinose and xylose production from arabinoxylan in corn hull and bagasse, respectively, both of which have different 1-L-arabinose contents.

MATERIALS AND METHODS

Materials. Corn hull (finer than 80 mesh) and bagasse (finer than 36 mesh) were obtained from Sanwa Cornstarches Ltd. (Japan, Nara) and Ajinomoto Ltd. (Japan, Tokyo), respectively. The starch residing in the corn hull was completely removed by enzymatic digestion with both 400 U a-amylase (*Bacillus subtilis* origin, Nagase Chemtex Co., Ltd.) and 170 U glycoamylase (*Aspergillus niger* origin, Nagase Chemtex Co., Ltd.) per gram of corn hull (wet weight). Hydrolysis of the starch was conducted at 60°C for 24 h in 0.1 M acetate buffer (pH 6.0) including 1.1 mM calcium acetate and 3.4 mM NaCl. Bagasse and the treated corn hull contain 45.0 and 21.8% cellulose, 26.0 (Ara/Xyl = 0.073) and 43.2% (Ara/Xyl = 0.590) arabinoxylan, and 19.6 and 10.9% Klaeson lignin, respectively. Commercial cellulase (Meicelase; *Trichoderma viride* origin, Meijiseika Ltd. Japan, Tokyo) was used to hydrolyze the corn hull. Standard xylobiose and xylotriose samples were purchased from Wako Pure Chemical Industries Ltd. for use in high performance liquid chromatography (HPLC) analysis. All other chemicals were reagent grade.

Heat-treatment with dilute HCl and enzymatic hydrolysis. For heat-treatment with water, 500 mg of corn hull was mixed with 5 mL of water in 10 mL test tubes. The tubes were then capped and put in a block heater at...
145–185°C for 20 min, then cooled down at room temperature. After centrifugation at 3000 rpm (1500×g) for 10 min the supernatant was subjected to sugar analysis on HPLC.

For heat-treatment with dilute acid, 50 mg of corn hull or bagasse and 0.5 mL of 0.2 M HCl were mixed in 10 mL test tubes. The tubes were then capped and heated at 125 or 130°C for 2–30 min in a block heater. After heating, they were cooled down at room temperature and the treated mixture was neutralized with 50 μL of 1 M NaOH. The pH value was adjusted to 5.0 by adding 0.45 mL of 0.1 M acetate buffer (pH 5.0). An enzyme solution (0.2 mL) was then added to the reaction mixture (1 mL). The substrate concentration was 4.5% by weight of corn hull or bagasse before the dilute-acid treatment and the enzyme concentration was 1.8 mg/mL (10.8 U/mL for carboxymethyl cellulose). After incubation at pH 5 and 40°C for 24 h with stirring, the mixture was centrifuged at 3000 rpm (1500×g) for 10 min, and the supernatant was applied to sugar analysis on HPLC.

Analysis of the sugars. Sugars released from the corn hull and bagasse were analyzed by HPLC under the following conditions: column, GL-C610 (250×7 mm ID, Hitachikasei Ltd.); mobile phase, water; column temperature, 60°C; flow rate, 1.0 mL/min; and detector, Hitachi model L-3300 differential refractive index monitor.

RESULTS AND DISCUSSION

Heat-treatment with water alone was attempted to release L-arabinose and xylose from corn hull, which have higher arabinoxylan (L-arabinose/xylose =0.590) and lower lignin contents than those of bagasse. Figure 1 shows the relationship between the released sugar amounts per 1 gram of corn hull and the treatment temperature. With heat-treatment, L-arabinose was more copiously released than xylose. This suggests that arabinoxylan can be degraded at high temperatures and that L-arabinose of the side chain in xylan is more easily released. However, the amount of L-arabinose (less than 78 mg/g corn hull) was very small and almost constant at 170–180°C in this treatment alone. The small sugar recovery illustrates that the release of L-arabinose is decreased by secondary degradation at higher temperatures. Heat-treatment at lower temperatures is therefore necessary for more efficient L-arabinose release because of depression of secondary degradation.

Next, dilute acid was added to the heat-treatment at low temperature. Figure 2A shows the changes in the amount of sugars released by treatment with 0.2 M HCl at 125°C as a function of the treatment time. Hydrochloric acid catalyzed the hydrolysis of arabinoxylan at lower temperatures, and the amounts of L-arabinose and xylose released were greatly improved during the treatment. L-Arabinose was more preferentially released than xylose over a much shorter treatment period (less than 5 min). As shown in Table 1, treatment for 5 min resulted in an L-arabinose yield of 73% from arabinoxylan, and the L-arabinose ratio in the released xylose and L-arabinose mixture was maximum (60%). The production of L-arabinose and xylose...
reached up to 162 and 290 mg/g corn hull, and the yields from arabinoxylan were 89 and 99%, respectively, after 20 min of the treatment. It was found that treatment with dilute hydrochloric acid was very effective in releasing monosaccharides from arabinoxylan. Other sugars of xylotriose, xylobiose and glucose were released but in very small amounts. Enzymatic methods have been tried to release L-arabinose from arabinoxylan. L-Arabinofuranosidase from many organisms that separated from nature. With enzymatic hydrolysis, however, it is difficult to completely release L-arabinose from arabinoxylan with high L-arabinose content such as corn hull. The high content results in inhibiting enzyme attack because of steric hindrance between the L-arabinose side chains on the xylan main chain. Finally, the enzyme actions of both L-α-arabinofuranosidase and xylanase are depressed and the sugar yields are limited.

After the treatment with 0.2 M HCl at 125°C, the corn hull residues were applied to enzymatic hydrolysis with cellulase. Figure 2B shows the relationship between the amounts of sugars released and the treatment time. The amount of glucose released greatly increased with the treatment time and the effect was sufficient at more than 5 min. This was attributable to the pretreatment effect of hydrochloric acid on enzymatic hydrolysis with cellulase. The cellulase affinity to cellulose was improved by removal of the rigid arabinoxylan coverage area, and as shown in Table 2, the yield from cellulose was improved up to 79−83%. In general, cellulose has a crystalline structure that is not easy to break down with pretreatment at low temperature. The high yield of glucose supports the suggestion that the structure of cellulose in corn hull is not as rigid; that is, it is relatively less crystalline than other species. Furthermore, corn hull has lower lignin content, which prevents enzymes from attacking cellulose.

Figure 3A shows the effect of the dilute-acid treatment (0.2 M HCl, 130°C) on bagasse. Large amounts of xylose were released, remaining almost constant for more than 15 min. As bagasse arabinoxylan has a low L-arabinose content, the amount of L-arabinose released was much smaller than that from corn hull. As shown in Fig. 3B, enzymatic hydrolysis with cellulase after the treatment resulted in an almost constant release of glucose for more than 5 min. As shown in summary of the results of 30 min-treatment in Table 3, the pretreatment effect for the enzymatic hydrolysis of bagasse was limited to 26% yields for cellulose and not dependent on the treatment time over 5 min. Although dilute-acid treatment led to an L-arabinose yield of 99% from arabinoxylan, xylose yield was smaller (63%) and the release of glucose by enzymatic hydrolysis with cellulase was not improved. Bagasse has a more rigid structure than corn hull because of the higher lignin content of 19.6%. Lignin covers cellulose and xylan and inhibits the enzymatic reaction. Although treatment with a higher concentration of hydrochloric acid at a higher temperature is necessary to produce large sugar recovery, L-arabinose and xylose yields from arabinoxylan of hemicellulose decrease with overly severe treatment because of secondary degradation, where,
both L-arabinose and xylose were obtained and these sugars can be separated by chromatographic methods such as cation-exchange chromatography.

This paper is dedicated to my (M.K.) mentor, the late Professor Dr. Toshiaki Komaki, Fukuyma University, in memory of their numerous pioneering works and leadership in the field of starch and its related enzyme science.

REFERENCES


トウモロコシ種皮およびバガスからの
L-アラビノースおよびキシロースの生産
倉掛昌裕，相知一也，木坂 涉，小卷利章
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125 または 130℃ での希塩酸処理によるトウモロコシ種
皮およびバガスのアラビノキシランからの L-アラビノー
スおよびキシロースの生産性について調べた。L-アラビ
ノース含量の高いアラビノキシランを含むトウモロコシ
種皮（Ara/Xyl=0.590）では、0.2 M HCl で 125℃ 处理に
おいて、処理時間 5 分以内で、L-アラビノースがキシ
ロースより優先的に遊離した。5 分間処理での収率は
73% であったが、その L-アラビノース組成は 60% と高い
ものであった。20 分処理においての L-アラビノースおよ
びキシロース生成量は 162 および 290 mg/g corn hull であ
り、アラビノキシランからの収率はそれぞれ 89 および
99% であった。さらに処理後のセルラーゼ剤による加水
分解でのグルコース生成量は非常に高かった。他方、L-
アラビノース含量の低いアラビノキシランを有するバガ
スでは、処理による L-アラビノース収率は 99% であった
が、リグニンを多く含むためキシロース収率は 63% で、
酵素反応によるグルコース生成量も低かった。

コメント：この論文は小卷先生とともにトウモロコシ
の種皮の利用について初めて行った実験で、非常に思い
出深いものです。この後、酵素による L-アラビノースの
生産法の研究に進むことになりました。また、この実験
に熱心に取り組んでいた当時卒研生だった相知一也君も
小卷先生と同じ年に病気で亡くなりました。お二人のご
冥福をお祈り申し上げ、この論文を捧げたいと思います。