コーンファイバーの部分酸加水分解によるL-アラビノースの生産

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Partial Acid Hydrolysis of Corn Fiber for the Production of L-Arabinose

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Conditions of acid hydrolysis for the preferential liberation of L-arabinose from corn fiber, a co-product of corn wet-milling processes, were investigated for the purpose of studying the production of L-arabinose. Prior to hydrolysis, starch was removed from the fiber by treatment with α-amylase. L-Arabinose was liberated rapidly at the beginning of hydrolysis and then slowed when the yield reached 12-13%. Conversely, the liberation of D-xylose was slow but linearly increased to more than 20% yield. Approximately 50 to 60% of L-arabinose in the destarched corn fiber (DSCF) was preferentially released with 0.2 N oxalic acid or 0.1 N sulfuric acid at 100℃ for 3 h. The ratio of L-arabinose to D-xylose released was characteristic by the kind of acid used. Oxalic acid was the best for the production of L-arabinose, and hydrochloric acid was good for the production of D-xylose. Oligosaccharides were also produced in these hydrolyses. The amounts and degree of polymerization (DP) of the oligosaccharides yielded with 0.1 and 0.4 N oxalic acid at 100℃ for 1 h were 43%, DP 15.8, and 38%, DP 7.9, respectively. The suitable conditions so far examined for the preferential liberation of L-arabinose were hydrolysis with 0.3-1.0 N oxalic acid at 100℃ for 1 h, which produced 15% (62% of all L-arabinose in DSCF) L-arabinose. The yields of L-arabinose, D-xylose and soluble oligosaccharides could be controlled by the conditions of hydrolysis.

Large amounts of cell wall materials such as, corn fiber, wheat bran, potato fiber, and beet pulp are produced by agricultural product processing industries. They are mainly used in animal feed or burned as fuel, although small amounts of them are refined and used as dietary fiber.

The wet-milling corn starch industry supplies 80% of the starch demand in Japan, approximately 2.4 million tons a year. It produces 252,000 tons of fiber fraction as a coproduct. The main component of the fiber fraction is arabinoxylan, a hemicellulose. The purpose of this study is to find a novel use for this arabinoxylan. One of the main constituents of arabinoxylan is L-arabinose. It is known that the sugar has a unique function: to inhibit small intestinal sucrase[1-3] and consequently, to decrease the digestion and absorption of sucrose.[2,3] Furthermore, almost no L-arabinose is absorbed from the small intestine in rats,[4] chicks,[5,6] and pigs.[7] L-Arabinose is not metabolized in humans.[8] It is sweet, similar to sucrose but with half the sweetness. Sucrose is the favorite nutritive sweetener, but too much ingestion triggers obesity and such effects as diabetes and cardiovascular diseases,[9] and it is a serious problem in developed countries. This disadvantage may be covered by ingestion with a small amount of L-arabinose. L-Arabinose

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can be produced by the acid hydrolysis of some plant gums such as mesquite, cherry, black wattle, and ghatti and from some agricultural by-products such as wheat or rye bran,[10,11] but it appears to be expensive as a foodstuff. In this paper, we investigated the process to produce L-arabinose from corn fiber by acid hydrolysis.

MATERIALS AND METHODS

Pretreatment of corn fiber. Corn fiber was treated with α-amylase to remove starch prior to hydrolysis. Corn fiber (400 g) was suspended in 2 L of 0.08 M phosphate buffer, pH 6.0, homogenized for 1 min at 20,000 rpm and heated for 15 min at 90℃. Commercial α-amylase (1.2 mL, termamyl 120 L, Novo Nordisk, 120 KNU/g, 1.2 g/mL) was added to the suspension and starch was digested for 1 h. The resulting sugars were washed out with excess distilled water on a 22 μm mesh screen, and the destarched corn fiber (DSCF) was dried at 60℃ for 2 days.

Acid hydrolysis. DSCF (1 g) was hydrolyzed at 100℃ for 0.5-6.0 h in 10 mL (final volume) of 0.01-0.1 N hydrochloric acid, 0.05-0.5 N sulfuric acid, or 0.01-2.0 N oxalic acid. The hydrolyzates were cooled in running water for 10 min and neutralized with sodium hydroxide for hydrochloric acid, barium hydroxide for sulfuric acid, and calcium chloride for oxalic acid to precipitate sulfate and oxalate, respectively. The neutral suspension was made up to 25 mL with distilled water and centrifuged (3000×*g*, 10 min).

Estimation of cellulose and the sugar composition of corn fiber. Cellulose and the sugars in the DSCF were estimated by the methods of Englyst *et al.*[12] with minor modifications. DSCF (600 mg, dry basis) was treated with 2 mL of 24 N sulfuric acid for 1 h at 35℃, diluted 12-fold with distilled water and hydrolyzed at 100℃ for 1 h. The hydrolyzate was neutralized with barium chloride (5.9 g) and 2 N sodium hydroxide (24 mL), made up to 100 mL, and the reaction mixture was centrifuged at 3000 rpm for 10 min. The resulting D-glucose was determined by high-performance anion exchange chromatography (HPAEC) and cellulose was estimated by the amount of glucose assuming it originated only from cellulose.

The sugar composition of noncellulose components was analyzed after mild acid hydrolysis.[12] DSCF (600 mg, db) was hydrolyzed with 20 mL of 2 N sulfuric acid at 100℃ for 2 h, neutralized with barium chloride (4.9 g) and 2 N sodium hydroxide (20 mL), increased to 100 mL with distilled water, and centrifuged at 3000 rpm for 10 min. Soluble sugars were determined by HPAEC or high-performance liquid chromatography (HPLC).

HPAEC analysis. HPAEC was performed by a DX 500 chromatography system (Dionex) equipped with an autosampler AS-3500 and a Peak Net System installed in SWHJ2 (Dell) as the controller and calculator of peak areas. The working and reference electrodes were gold and silver-silver chloride, respectively. The pulse potentials and durations at a range of 10 μC were as follows: $E_1=0.05$ ($t_1=400$); $E_2=0.75$ ($t_2=200$); and $E_3=-0.15$ V ($t_3=400$ ms). Columns of CarboPac PA1 Gard (4×50 mm, Dionex) and CarboPac PA1 (4×250 mm, Dionex) were connected in series. Eluents A, B, and C were distilled water, 100 mM sodium hydroxide, and 100 mM sodium hydroxide containing 1 M sodium acetate, respectively. The baselines of chromatograms were kept stable by using a post-column system with the addition of 300 mM sodium hydroxide to an Anion Micromembrane Suppressor (AMMS-II). Each eluent was prepared with 18 MΩ cm deionized and distilled water and filtered through a 0.2 μm membrane filter. Runs were carried out at ambient conditions with a flow rate of 1.0 mL/min by the gradient program summarized in Table 1.

HPLC analysis. HPLC was carried out by an HPLC system with a Chromatocorder 21 (SIC) using an Ionpak S-801 column (8×500 mm, Shodex) connected to an Ionpak S-800P (8×50 mm, Shodex) maintained at 80℃. Eluent water was flowed at a rate of 1.5 mL/min, and monitored by a differential refractometer (RI, Jusco RID 300).

Size-exclusion chromatography (SEC). The hydrolyzates of DSCF with oxalic acid (0.1 N, 0.2 N, and 0.4 N) were subjected to SEC using

Table 1. Gradient program for HPAEC.

Time (min)	D. Water	0.1 M NaOH	0.1 M NaOH/1 M AcONa
0.0	90.0	10.0	0.0
0.9	90.0	10.0	0.0
1.0	100.0	0.0	0.0
40.0	100.0	0.0	0.0
45.0	90.0	10.0	0.0
65.0	17.5	80.0	2.5
85.0	10.0	87.0	3.0
100.0	0.0	96.5	3.5
120.0	0.0	93.5	6.5
137.3	0.0	87.0	13.0
150.0	0.0	0.0	100.0
165.0	0.0	0.0	100.0

Bio-Gel P-2 (30×290 mm) to separate the oligosaccharides from monosaccharides. The hydrolyzed specimen (5 mL) filtered through a 0.44 μm membrane filter was applied on Bio-gel P-2. The eluent water was flowed at a rate of 40 mL/h at ambient temperature, and 4 mL fractions were collected.

Other methods. Total carbohydrate and reducing sugars such as D-xylose were determined by the methods of phenol-sulfuric acid[13] and Somogyi-Nelson,[14,15] respectively.

Reagents. Chemicals were of the highest grade commercially available.

RESULTS

Removal of starch from corn fiber.

Commercial corn fiber (Sanwa Cornstarch Co., Nara) contained approximately 15% starch, which produces D-glucose by acid hydrolysis, and D-glucose may interfere with the separation and purification of L-arabinose and D-xylose from the hydrolyzate. Therefore, corn fiber was treated with α-amylase (Termanyl 120 L) to remove the starch. The removal of starch was complete because no detectable D-glucose was found by means of Glu LB (Wako Co., Ltd.) after the treatment of the fiber with amyloglucosidase (Sigma), and the DSCF was used for further studies.

Monosaccharide composition of DSCF.

The sugar composition of hemicellulose and the amount of cellulose are shown in Table 2. Some amounts of sugars could be degraded during acid hydrolysis, but their degradation was supposed to be the same. Similar values were reported by Saulnier *et al.* in 1995.[16] The content of L-arabinose in DSCF was found to be approximately 24%.

Conditions for the preferential liberation of L-arabinose by acid hydrolysis.

To find the suitable conditions for hydrolysis, DSCF was hydrolyzed with varied concentrations of oxalic, sulfuric, and hydrochloric acids at 100℃ for 1 h, and the sugars liberated were analyzed by HPAEC and/or HPLC. The results are shown in Figs. 1, 2 and 3. L-Arabinose (11.5%) and D-xylose (2.7%), and those of 16.8 and 21.6%, were released from DSCF with 0.2 N and 2.0 N oxalic acid, respectively (Fig. 1). Very few amounts (>0.4%) of glucose were produced (probably from cellulose) with higher concentrations than 2.0 N oxalic acid. Hydrolysis with concentrations higher than 0.4 N oxalic acid also released 4–5% of D-xylobiose. Neither D-mannose nor D-galactose was found under these conditions. The major soluble components of the products by sulfuric (Fig. 2) and hydrochloric (Fig. 3) acids at lower concentrations were also L-arabinose and D-xylose. In these hydrolyses,

Table 2. Sugar composition of DSCF.

Ara	Xyl	Gal	Glc[a]	Man	Glc A	Others[b]	Total	Reference
27.0 (24.3)[c]	33.8 (30.4)	7.6 (6.8)	29.5 (26.7)	1.1 (1.0)	1.0 (0.9)	—	100 (90)	This study
22.8	34.3	5.6	22.5	—	4.8	10	100	16

[a]Originated from cellulose (hydrolysis by strong acid, see text). [b]Includes protein, ferulic acid, and diferulic acid, and these were not determined in this study. [c]The value corrected for others (assumed as 10%).

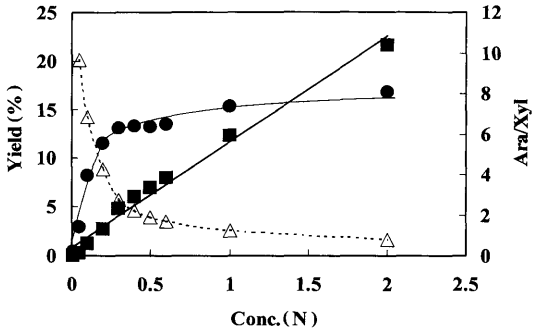

Fig. 1. The liberation of L-arabinose and D-xylose from DSCF by hydrolysis with oxalic acid.

Conditions: 100℃ for 1 h. The circles and squares are the liberated L-arabinose and D-xylose, respectively. The triangles are the ratio of L-arabinose to D-xylose.

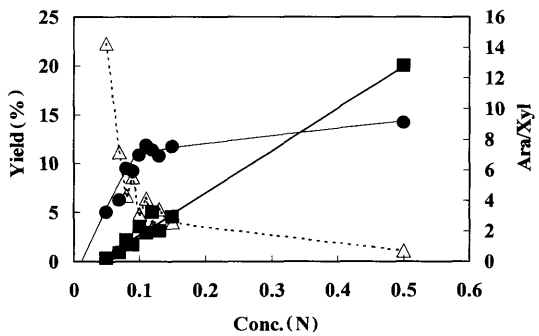

Fig. 2. The liberation of L-arabinose and D-xylose from DSCF by hydrolysis with sulfuric acid.

Conditions and symbols: see Fig. 1.

the yields of D-xylose were increased linearly with increases in acid concentration; however, the rate of L-arabinose production slowed when it reached 10–13% of DSCF, indicating the limit as shown in Figs. 1, 2 and 3. It is obvious that the production of L-arabinose is much faster than that of D-xylose, and oxalic acid appears to be the choice acid for preferential production of L-arabinose.

To examine the detail conditions for the preferential liberation of L-arabinose, the hydrolyses were carried out using low concentrations (0.05–0.2 N) of oxalic acid and for prolonged periods (0.5–6.0 h). The yields of L-arabinose and other soluble products are listed in Table 3. Besides L-arabinose, small amounts of xylobiose were liberated with 0.1 and 0.2 N

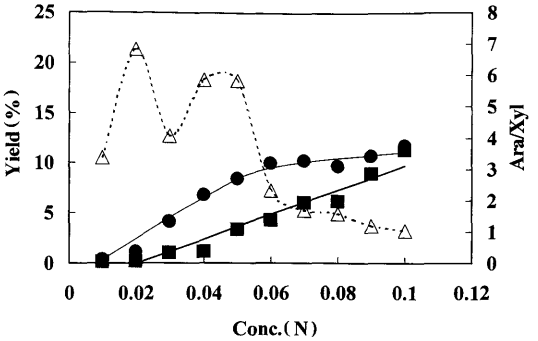

Fig. 3. The liberation of L-arabinose and D-xylose from DSCF by hydrolysis with hydrochloric acid.

Conditions and symbols: see Fig. 1.

oxalic acid for more than 2 h, and the yield continuously increased throughout the period. The yield of L-arabinose also increased by prolonged hydrolysis with 0.05–0.20 N oxalic acid, but 13% was the maximum. The yields of soluble components by hydrolysis with 0.08–0.12 N sulfuric for 0.5–6.0 h are summarized in Table 4. The yields of L-arabinose and other soluble products, but not xylobiose and cello-oligosaccharides, were similar to those with oxalic acid, but the concentration of sulfuric acid was lower than that of oxalic acid to attain similar levels of hydrolysis to oxalic acid because of the strong acid. Almost undetectable amounts of xylobiose and cellooligosaccharides were found in the case of sulfuric acid (Table 4).

Analysis of oligosaccharides.

Some oligosaccharides were also produced by partial acid hydrolysis. Their amounts and sizes were analyzed by size-exclusion chromatography. Figure 4 shows the chromatograms of the products obtained by hydrolysis with 0.1 N oxalic acid at 100℃ for 1 h. In the case of hydrolysis with 0.1 N oxalic acid, the peak height of the fraction (L) corresponding to oligosaccharides was slightly higher than that of the fraction (S) of small molecules (monosaccharides) (Fig. 4). In the case of hydrolysis with 0.4 N oxalic acid, the peak height of fraction S was higher than that of fraction L, and in the case of 0.2 N acid, the peak heights of fractions L and S were nearly the same (the

Table 3. Time courses of hydrolysis with oxalic acid.

	Arabinose (%)[a]	Xylose (%)[a]	Xylobiose (%)[a]	Cellooligo[b] (%)[a]	Others[c] (%)[a]
0.05 N					
0.5 h	1.5	0.2	0.0	0.0	6.2
1.0 h	2.9	0.3	0.0	0.2	8.0
2.0 h	4.6	0.4	0.0	0.0	11.2
3.0 h	6.2	0.8	0.0	0.0	14.6
6.0 h	8.4	1.4	0.0	0.0	19.1
0.10 N					
0.5 h	5.4	0.6	0.0	0.1	15.8
1.0 h	8.2	1.2	0.0	0.0	18.9
2.0 h	11.3	3.1	3.8	1.4	38.9
3.0 h	12.2	5.7	1.7	0.6	30.4
6.0 h	12.2	5.9	4.0	1.5	33.2
0.20 N					
0.5 h	9.8	2.1	0.0	0.0	22.5
1.0 h	11.5	2.7	0.0	0.5	32.8
2.0 h	11.2	3.4	1.7	0.8	36.7
3.0 h	12.3	8.3	4.2	2.4	34.4
6.0 h	13.0	14.6	4.7	1.9	27.5

[a]DSCF (dry weight) as 100%. [b]Cellobiose, cellotriose, cellotetraose, cellopentase, cellohexaose. [c]The peaks were detected at shorter periods than those of cellohexaose, calculated as xylose.

Table 4. Time courses of hydrolysis with sulfuric acid.

	Arabinose (%)[a]	Xylose (%)[a]	Xylobiose (%)[a]	Cellooligo[b] (%)[a]	Others[c] (%)[a]
0.08 N					
0.5 h	7.3	1.5	0.0	0.0	18.0
1.0 h	9.5	2.2	0.0	0.0	23.8
2.0 h	11.5	5.6	0.0	0.0	34.5
3.0 h	11.1	7.6	0.0	0.0	34.6
6.0 h	11.9	13.9	0.0	0.0	32.4
0.10 N					
0.5 h	7.9	1.5	0.0	0.0	20.5
1.0 h	10.8	3.5	0.0	0.0	31.2
2.0 h	11.8	8.4	0.0	0.2	38.5
3.0 h	12.3	11.9	0.0	0.0	34.8
6.0 h	12.5	19.4	0.0	0.0	74.6
0.12 N					
0.5 h	9.9	3.2	0.0	0.0	41.8
1.0 h	11.4	5.0	0.0	0.0	58.6
2.0 h	11.8	11.8	0.0	0.0	64.5
3.0 h	12.3	16.3	0.0	0.0	69.3
6.0 h	13.1	29.4	0.0	0.0	78.1

[a]DSCF (dry weight) as 100%. [b]Cellobiose, cellotriose, cellotetraose, cellopentase, cellohexaose. [c]The peaks were detected at shorter periods than those of cellohexaose, calculated as xylose.

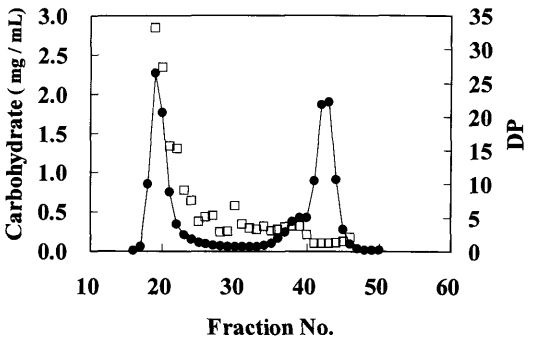

Fig. 4. Gel permeation chromatogram of the soluble fraction produced with 0.1 N oxalic acid hydrolysis for 1.0 h on Bio-Gel P-2.

Circle, carbohydrate; square, DP.

chromatograms are not shown). These results suggest that the oligosaccharides and monosaccharides were the major components of the soluble products in 0.1 and 0.4 N hydrolyzate, respectively. These two fractions, L and S, and fraction M (the valley of the L and S fractions) were separately collected, and the amounts and DP of these fractions were analyzed (Table 5). The weight proportions of L were decreased and those of S increased with increasing concentrations of acid. The whole amounts of the carbohydrates liberated with the 0.4 N acid were 1.45-fold higher than those with 0.1 N acid. The DP of solubilized DSCF by oxalic acid-hydrolysis decreased with increased acid concentration. The DP values of fraction L yielded with 0.1, 0.2 and 0.4 N acid were 15.8, 11.3 and 7.9, respectively, suggesting that they were mixtures of fairy large oligosaccharides. The DP values of fraction S were in the range of 0.7-1.2, indicating mainly monosaccharides, a mixture of L-arabinose and D-xylose. Fraction M appeared to be a mixture of small oligosaccharides and small amounts of L-arabinose and D-xylose.

DISCUSSION

We investigated the possibility of the production of L-arabinose from a low or almost no-value material of the corn-fiber fraction produced by the wet-milling corn starch processing industry through a simple process of acid hydrolysis. It is well known that the L-arabinofranosyl linkages in arabinoxylan found in corn, wheat, and other cereal brans are easily hydrolyzed with acid. Enzymic hydrolysis by arabinofuranosidases[18-23] [EC 3.2.1.55] together with some other hemicellulases may be used for the production of L-arabinose, and this is left for future studies.

The nonstarch fiber fraction includes 10-13% protein, 20-22% cellulose, 3-4% uronic acids,

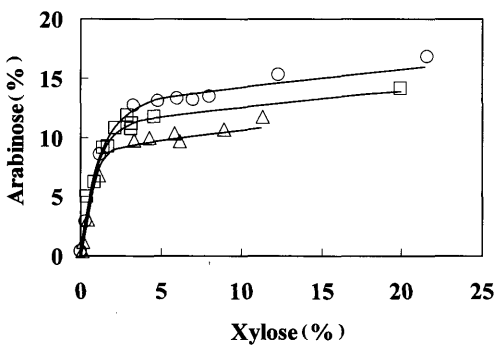

Fig. 5. Relative production of L-arabinose and D-xylose by hydrolysis with oxalic, sulfuric, and hydrochloric acids.

Circle, oxalic acid; square, sulfuric acid; triangle, hydrochloric acid.

Table 5. Carbohydrate amounts and DP of the chromatographed fractions of oxalic acid-hydrolyzed DSCF.

Conc (acid)	Carbohydrate								DP			
	L[a]		M[a]		S[a]		Whole		L	M	S	Whole
	mg[b]	%	mg[b]	%	mg[c]	%	mg[b]	%				
0.1 N	26	43	10	17	24	40	60	100	15.8	2.5	0.7	2.8
0.2 N	27	39	17	24	26	37	70	100	11.3	2.7	0.8	1.9
0.4 N	33	38	14	16	40	46	87	100	7.9	2.8	1.2	1.7

[a]L, M, S, see text. [b]Determined as xylose. [c]Determined as a mixture of L-arabinose and D-xylose based on HPLC analysis.

2-3% lipid and 2% ash, and 37% on average of the fiber is arabinoxylan, of which 5-14% is extractable by some alkalis.[17] The purification of arabinoxylan by alkali extraction before hydrolysis may be advantageous, but the yield of the arabinoxylan was low and expenses were high; therefore, we suggest using only destarched fiber.

The partial hydrolysis of DSCF under mild conditions by acids yielded preferentially L-arabinose, followed by D-xylose. At most, ~62% L-arabinose of the whole contents of DSCF could be released preferentially by hydrolysis with oxalic and sulfuric acids. The releasing rate of L-arabinose slowed down when the yield reached ~12%, which was ~44% of the total L-arabinose content of DSCF. L-Arabinose is mainly located in the nonreducing side-chains of arabinoxylan of corn bran. However, some ferulic acids, D-galactose and D-xylose have been suggested to be attached to the side-chain L-arabinose, which makes the arabinoxylan partly insoluble,[16] and these are considered to be the reasons for the slow release and incomplete liberation of L-arabinose.

The relationship between the production of L-arabinose and D-xylose by hydrolysis with oxalic, sulfuric, or hydrochloric acid is summarized in Fig. 5. The relative liberation of L-arabinose and D-xylose differed according to the kind of acid. Two possibilities may be considered as the reason for this difference. The first is the difference of the rate of liberation, and the second is the difference in the stability of the liberated sugars under the acidic conditions. Whether one or both are involved is not clear. Practically, however, oxalic acid appears to have an advantage for the production of L-arabinose, and hydrochloric acid may have an advantage for D-xylose production. Oxalic acid is easy to remove as an insoluble calcium oxalate by neutralization with calcium hydroxide, which is an another advantage for this process.

Acid hydrolysis is a very simple method for the preparation of mono- and oligosaccharides from hemicellulose, so the yields of the products, the ratio of L-arabinose to D-xylose, and the molecular sizes of oligosaccharides could be easily controlled by choosing conditions such as the kind and concentration of acid and the period and temperature of hydrolysis.

The present investigation suggests that L-arabinose together with D-xylose and soluble oligosaccharides are easily produced by the mild hydrolysis of corn fiber. D-Xylose and soluble oligosaccharides are also useful materials such as for sweetner and soluble dietary fiber, respectively. The conditions for the industrial production of L-arabinose may be chosen by considering the yields of L-arabinose, D-xylose and oligosaccharides and also the efficiency of the process for the following separation and purification of L-arabinose and other products from the hydrolyzate.

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コーンファイバーの部分酸加水分解によるL-アラビノースの生産

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トウモロコシ澱粉製造時の副産物，コーンファイバーからL-アラビノースを生産することを目的として，優先的にL-アラビノースを生成する酸分解の諸条件を検討した．酵素処理により脱澱粉したコーンファイバー (DSCF) を，酸の種類を選定するために稀薄なシュウ酸 (0.05-0.2 N)，硫酸 (0.08-0.12 N)，塩酸 (0.03-0.07 N) で，100℃，1-6時間加水分解すると，DSCFから最高13%のL-アラビノースが遊離した．これは，DSCF中のアラビノース含量の53%に相当した．L-アラビノースの生成は加水分解の初期に速やかであったが，生産量が10-12%に達すると遅くなった．一方，D-キシロースの生成はゆっくりと直線的に上昇した．DSCFの0.1-0.4 Nシュウ酸加水分解物の可溶成分を，バイオゲルP-2でオリゴ糖を分別し，その量と大きさを測定した．その結果，得られたオリゴ糖の量は38-43%，重合度は7.9-15.8であった．シュウ酸はアラビノースの生成に最も適した酸で，0.4 Nで100℃，60分の加水分解でDSCFから15%のL-アラビノースを生成した．一方，塩酸はD-キシロースの生産により適していた．酸の種類や濃度，反応時間の調整で，生成する単糖やオリゴ糖の量，重合度の制御が可能であることが示唆された．