

Compositional Analysis of the Oligosaccharide Units of Xyloglucans Isolated from the Cell Walls of Fruit Vegetables

主要果菜類細胞壁から分離したキシログルカンの
オリゴ糖単位の組成分析

Yoji KATO*, Teruko KONISHI*†, and Yasushi MITSUISHI**

加藤 陽治*・小西 照子*†・三石 安**

ABSTRACT

The oligosaccharide units of xyloglucans from some fruit vegetables were comparatively analyzed by enzymatic digestion followed by anion-exchange chromatography with pulsed amperometric detection. The enzymes used were a xyloglucan-specific endo-1,4- β -D-glucanase (xyloglucanase) from *Penicillium* sp. M 451 and an isoprimeverose-producing oligoxyloglucan hydrolase from *Eupenicillium* sp. M9. The oligosaccharide units of the polysaccharides were XXXG, XXLG, XLXG, XXFG, XLLG, and XLFG [where each (1→4)- β -linked D-glucosyl residue in the backbone is given a one-letter code according to its substituents: G, β -D-Glc; X, α -D-Xyl-(1→6)- β -D-Glc; L, β -D-Gal-(1→2)- α -D-Xyl-(1→6)- β -D-Glc; F, α -L-Fuc-(1→2)- β -D-Gal-(1→2)- α -D-Xyl-(1→6)- β -D-Glc] in an approximate molar ratio of 36 : 4 : 6 : 31 : 1 : 22 for cucumber, of 37 : 8 : 5 : 30 : 1 : 19 for mung bean sprouts, and of 35 : 4 : 7 : 25 : 4 : 25 for pumpkin.

* 弘前大学教育学部家政学科教室

Laboratory of Food Science, Department of Home Economics, Faculty of Education, Hirosaki University, (1 Bunkyo-cho, Hirosaki 036-8560, Japan)

** 工業技術院生命工学工業技術研究所

National Institute of Bioscience and Human Technology, AIST, (1-1 Higashi, Tsukuba 305-0046, Japan)

† 現在 京都大学木質科学研究所

Present address: Wood Research Institute, Kyoto University, (Gokasho, Uji, Kyoto 611-0011, Japan)

Abbreviations: UA, uronic acid; Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Glc, glucose; Gal, galactose; IP [α -D-Xyl-(1→6)-D-Glc], isoprimeverose; IPase, isoprimeverose-producing oligoxyloglucan hydrolase; HPAEC, high-performance anion-exchange chromatography; PAD, pulsed amperometric detection. FG, LG, LXG, LFG, LLG, XXXG, XXFG, XLFG, XLXG, XXLG, and XLLG are the abbreviated names for xyloglucan oligosaccharides according to the nomenclature of Fry *et al.* [ref.¹⁾ Each (1→4)- β -linked D-glucosyl residue in the backbone is given a one-letter code according to its substituents: G, β -D-Glc; X, α -D-Xyl-(1→6)- β -D-Glc; L, β -D-Gal-(1→2)- α -D-Xyl-(1→6)- β -D-Glc; F, α -L-Fuc-(1→2)- β -D-Gal-(1→2)- α -D-Xyl-(1→6)- β -D-Glc].

Key words: xyloglucan; xyloglucan oligosaccharide; fruit vegetables; cell walls

INTRODUCTION

Xyloglucan is the predominant hemicellulosic polysaccharide of the primary cell walls of dicotyledonous plants, typically forming 20 to 25% of dry mass of the cell wall.²⁾ A marked characteristic of the xyloglucan is that the polysaccharide consist of a linear backbone chain of 1,4-linked β -D-Glc residues and side chains of single α -D-Xyl residues, α -L-Fuc-(1 \rightarrow 2)- β -D-Gal-(1 \rightarrow 2)- α -D-Xyl residues, β -D-Gal-(1 \rightarrow 2)- α -D-Xyl residues, and others that are attached to the O-6-positions of the backbone chain. Treatment of xyloglucan with endo- β -1,4-glucanase generates mainly oligosaccharide subunits (XXXG, XLXG, XXLG, XLLG, XXFG, XLFG, and so on) of the xyloglucan polymer.³⁾

Xyloglucan polysaccharides and some oligosaccharides play a role in the regulation of plant growth.⁴⁻⁶⁾ Furthermore, xyloglucan oligo- and polysaccharides were demonstrated to have some physiological effects on animals.⁷⁻⁹⁾ From this point of view, the compositional analysis of oligosaccharide units in xyloglucan polymer is very important. In the previous work, we characterized the oligosaccharide units of xyloglucans isolated from the cell walls of leaf and root vegetables.¹⁰⁾ The present work was conducted to elucidate the composition of oligosaccharide units of xyloglucans isolated from the cell walls of commercially available fruit vegetables.

MATERIALS AND METHODS

Cell wall polysaccharides — The cell wall polysaccharides of cucumber, mung bean sprouts and pumpkin were fractionated respectively into four fractions, PS (pectic substance), HC (hemicellulose)-I, HC-II, and CL (cellulose) fractions, by using successive extraction with 0.5% ammonium oxalate, 4% KOH, and 24% KOH, and subsequent dialysis of the individual extracts as described in previous papers.¹¹⁾ The HC-II fractions (24% KOH extracts)¹¹⁾ were used for the present work.

Resolution of HC-II fractions — Each HC-II fraction (18.0 mg for cucumber, 21.2 mg for mung bean sprouts, and 14.1 mg for pumpkin) was dissolved in 1 ml of 20 mM Na-acetate buffer (pH5.0), then centrifuged to remove insoluble material. The supernatant was applied to a column (1.8 \times 8 cm) of DEAE-Sephadex A-25 equilibrated with 20 mM Na-acetate buffer (pH5.0) and eluted stepwise with 45 ml of the same buffer, 63 ml of 1.0 M NaCl in the same buffer, and 45 ml of 0.5 M NaOH. Fractions of 3.0 ml each were collected and assayed for carbohydrate by the phenol-H₂SO₄ method.¹²⁾ Tubes 4-10 were combined, dialyzed against distilled water, and freeze-dried to give HC-II-1 (xyloglucan) fraction. Yields of HC-II-1 fractions of cucumber, mung bean sprouts, and pumpkin were 9.5 mg, 12.1 mg, and 5.5 mg, respectively.

Sugar composition analysis of HC-II-1 fractions — HC-II-1 fractions (about 0.1 mg) were hydrolyzed with 2 M trifluoroacetic acid (TFA) for 4 h at 100°C. In each case the hydrolyzate was evaporated to dryness. Alditol trifluoroacetates derived from sugars were analyzed by gas liquid chromatography on a column (0.4 × 200 cm) packed with 1.5% QF-1 on Chromosorb W at 140°C.¹³⁾ Uronic acid was determined by the carbazole-H₂SO₄ method.¹⁴⁾

Iodine staining of HC-II-1 fractions — An aqueous solution of HC-II-1 (0.5 ml, containing about 0.2 mg) was combined with 0.5 ml of a solution containing iodine (0.5%, w/v) and potassium iodide (1%, w/v). A 2.5 ml solution of sodium sulfate (20%, w/v) was then added under shaking. After 1 h in the dark absorption of the reaction mixture at 450–800 nm was read in a photometer against a blank.¹⁵⁾

Methylation analysis of HC-II-1 fractions — Each sample (about 2 mg) in dimethylsulfoxide (0.5 ml) was methylated with methylsulfinyl carbanion (0.5 ml) and methyl iodide (0.5 ml) by the method of Hakomori.¹⁶⁾ After methylating, the reaction mixture was dialyzed against tap water, and the non-dialyzable materials were concentrated to dryness. The methylated HC-II-1 was hydrolyzed with 2 M TFA at 120°C for 1 h, the acid removed by evaporation. The methylated sugars were converted into their corresponding alditol acetates¹⁷⁾ and then analyzed by gas liquid chromatography in a glass capillary column (15 m × 0.32 mm) of DB-225. The column oven temperature was raised linearly from 140 to 200°C at a rate of 2°C/min.

Enzymatic hydrolysis of HC-II-1 fractions — A xyloglucan-specific endo-1,4-β-D-glucanase (xyloglucanase) from *Penicillium* sp. M451¹⁸⁾ and an isoprimeverose-producing oligoxyloglucan hydrolase (IPase) from *Eupenicillium* sp. M9¹⁹⁾, purified respectively to the electrophoretically pure state, were used.

Each HC-II-1 fraction (1 mg/ml 20 mM Na-acetate buffer, pH 5.5) was hydrolyzed at 40°C for 24 h with *Penicillium* sp. M451 xyloglucanase. A portion of xyloglucanase-treated xyloglucan (0.5 mg/0.5 ml 20 mM Na-acetate buffer, pH 5.5) were hydrolyzed at 40°C for 24 h with *Eupenicillium* sp. M9 IPase.

High-performance anion exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) — HPAEC-PAD analyses of oligosaccharides were performed on a Dionex chromatography system DX-300 (Dionex gradient pump, Dionex pulsed electro-chemical detector with a gold working electrode, and an Ag/AgCl reference electrode) interfaced with an AI-450 work station as described previously.²⁰⁾ Separations were performed at 20°C on a column (4 × 250 mm) of Dionex CarboPac PA 1 anion exchange resin with a CarboPack guard column, using a flow rate of 1 ml/min. Oligosaccharides were eluted with the following NaOAc gradient profile in 100 mM NaOH, 0–30 min, 0–50 mM; 30

-50 min, 50 mM; 50-60 min, 50-500 mM.

RESULTS AND DISCUSSION

Isolation and characterization of xyloglucans from cell walls of cucumber, mung bean sprouts, and pumpkin — The cell wall polysaccharides of cucumber, mung bean sprouts, and pumpkin were fractionated respectively into four fractions, PS, HC-I, HC-II, and CL fractions, by using successive extraction with 0.5% ammonium oxalate, 4% KOH, and 24% KOH, and subsequent dialysis of the individual extracts. The HC-II (24% KOH extracts) fractions consisted of UA, Rha, Fuc, Ara, Xyl, Man, Glc, and Gal in the molar ratio of 0.0 : 0.2 : 3.1 : 0.0 : 48.4 : 3.7 : 32.8 : 11.8 for cucumber, of 0.0 : 0.4 : 3.3 : 6.9 : 43.3 : 0.8 : 32.4 : 12.9 for mung bean sprouts, and of 0.0 : 1.5 : 1.6 : 3.8 : 48.0 : 1.0 : 23.7 : 20.6 as described in a previous paper¹¹⁾.

When HC-II fractions from cucumber, mung bean sprouts, and pumpkin were individually subjected to DEAE-Sephadex A-25 (acetate form) chromatography, they gave three subfractions, HC-II-1 (unbound fraction), -2 (fraction eluted with 1.0 M NaCl) and -3 (fraction eluted with 0.5 M NaOH). A neutral sugar composition analysis showed that HC-II-1 fractions consisted of Rha, Fuc, Ara, Xyl, Man, Glc, and Gal in the molar ratio of 0.0 : 5.7 : 0.6 : 34.0 : 10.6 : 38.1 : 10.9 for cucumber, of 0.0 : 5.0 : 1.7 : 39.4 : trace : 43.9 : 10.0 for mung bean sprouts, and of 0.0 : 4.1 : 0.5 : 31.5 : trace : 48.0 : 16.0 for pumpkin. Arabinose and mannose, were presumed to have arisen from contaminating pectic substances (arabinan and/or arabinogalactan) and mannanlike polysaccharides, respectively, which are commonly found in dicots. When the absorption spectra of the iodine complexes of HC-II-1 fractions were measured in the range of 450 to 800 nm, all HC-II-1 fractions showed an absorption peak at about 640nm, indicating that the xyloglucan was present in them.¹⁵⁾

Each HC-II-1 fraction was methylated and hydrolyzed, and the sugar linkage composition was analyzed by gas liquid chromatography of the resulting alditol acetates. 2,3,4-Tri-*O*-methyl-Xyl, 2,3,4-tri-*O*-methyl-Fuc, 2,3,4,6-tetra-*O*-methyl-Gal (and/or 3,4-di-*O*-methyl-Xyl), 3,4,6-tri-*O*-methyl-Gal, 2,3,6-tri-*O*-methyl-Glc, and 2,3-di-*O*-methyl-Glc were detected as major structural units in each methylated HC-II-1 fraction (Table 1), which suggests the possibility that each xyloglucan had a linear chain of β -1,4-D-Glc residues in the backbone, about 75% of which were branched at the *O*-6 positions, with single α -D-Xyl, β -D-Gal-(1 \rightarrow 2)- α -D-Xyl, and α -L-Fuc-(1 \rightarrow 2)- β -D-Gal-(1 \rightarrow 2)- α -D-Xyl residues. From these preliminary experimental results, the major polysaccharide in the respective HC-II-1 fraction was found to be a xyloglucan.

Table 1. Sugar-linkage composition of the HC-II-1 fractions obtained from the cell walls of cucumber, mung bean sprouts, and pumpkin.

Peak No.	Methylated sugar*	Deduced glycosidic linkage**	Amount (%total area)		
			Cucumber	Mung bean sprouts	Pumpkin
1	2,3,4-Me ₃ -Xyl	T-Xyl	11.2	17.2	8.4
2	2,3,4-Me ₃ -Fuc	T-Fuc	2.9	3.0	2.0
3	2,3,4,6-Me ₄ -Gal and/or 3,4-Me ₂ -Xyl	T-Gal and/or 2-Xyl	15.6	13.7	17.7
4	3,4,6-Me ₃ -Gal	2-Gal	5.5	4.9	8.0
5	2,3,6-Me ₃ -Glc	4-Glc	16.8	15.6	15.9
6	2,3-Me ₂ -Glc	4,6-Glc	47.9	45.5	48.0

* 2,3,4-Me₃-Xyl = 2,3,4-tri-*O*-methyl-1,4-di-*O*-acetyl-xylitol, etc.

** The numerical prefixes represent the carbon atoms involved in glycosidic linkages in the original polysaccharides. Prefix T indicates sugars linked through C-1 only.

Comparative analysis of the oligosaccharide units of xyloglucans from cucumber, mung bean sprouts, and pumpkin — Each HC-II-1 fraction (xyloglucan) was hydrolyzed with *Penicillium* sp. M451 xyloglucanase. A portion of xyloglucanase-treated xyloglucan was subjected to HPAEC-PAD. The residual xyloglucanase-treated xyloglucan were hydrolyzed with *Eupenicillium* sp. M9 IPase, and the resultant reaction mixture was subjected to HPAEC-PAD analysis. Figure 1 shows the case of cucumber xyloglucan. Peaks 1,2,3,4, and 5 found in Fig.1-A were identified to be XXXG, XXFG, XLXG and/or XLFG, XXLG, and XLLG. The approximate ratio of XXXG, XXFG, XLXG and/or XLFG, XXLG, and XLLG are 35.5 : 31.4 : 28.7 : 3.5 : 0.9, which were calculated from the peak area. Figure 1-B shows peaks 1 to 7 corresponding to Glc, IP, FG, LG, LXG, LFG, and LLG. It is well known that the IPase digestion of XXXG, XXLG, XLXG, XXFG, XLLG, and XLFG results in the production of IP and Glc (in the molar ratio of 3 : 1), IP and LG (2 : 1), IP and LXG (1 : 1), IP and FG (2 : 1), IP and LLG (1 : 1), and IP and LFG (1 : 1).²³⁾ The oligosaccharides found in Fig.1-B are certainly derived from xyloglucan oligosaccharides (XXXG~XLLG) found in Fig.1-A. LXG and LFG, which are characteristic IPase-digestion products of XLXG and XLFG, are found to be present in a ratio of 22 : 78. Cucumber xyloglucan was shown to be made up from XXXG:XXLG:XLXG:XXFG:XLLG:XLFG = 36 : 4 : 6 : 31 : 1 : 22.

In the same manner, the molar ratio of oligosaccharide units of other xyloglucans was calculated as shown in Table 2. The ratios of the six oligosaccharide units are slightly different among cucumber, mung bean sprouts, and pumpkin. However, the xyloglucans of cell walls of these vegetables are considered to be similar to each other in basic structure.

Tamarind seed xyloglucan has been shown to consist of four repeating oligosaccharide units (XXXG:XLXG:XXLG:XLLG = 13 : 9 : 28 : 50, mol%).²⁴⁾ The presence of XLLG in fucose-containing xyloglucans has not been reported. Most recently, we have reported that cabbage, Chinese cabbage, spinach, chingentsuai, lettuce, turnip, Japanese radish, edible burdock, carrot, and East Indian lotus xyloglucans are composed of XXXG, XXLG, XLXG,

XXFG, XLLG, and XLFG in 35 : 6 : 5 : 22 : 3 : 29, 35 : 9 : 5 : 24 : 2 : 25, 38 : 6 : 5 : 22 : 5 : 24, 35 : 14 : 6 : 19 : 4 : 22, 33 : 7 : 4 : 31 : 3 : 22, 35 : 3 : 8 : 17 : 4 : 33, 34 : 2 : 10 : 13 : 3 : 38, 34 : 3 : 4 : 27 : 4 : 28, 33 : 7 : 4 : 29 : 1 : 26, and 41 : 2 : 5 : 37 : 2 : 13, respectively.¹⁰⁾ Thus most fucose-containing xyloglucans may have essentially a small quantity of XLLG, XLXG, and XXLG besides XXXG, XXFG, and XLFG as major structural oligosaccharide units.

Fig. 1. HPAEC of xyloglucanase-hydrolyzate of cucumber xyloglucan (A) and of IPase-hydrolyzate of xyloglucanase-treated cucumber xyloglucan (B).

The retention times of peaks 1 to 5 of xyloglucanase-hydrolyzate of cucumber xyloglucan and of peaks 1 to 7 of IPase-hydrolyzate of xyloglucanase-treated cucumber xyloglucan were compared with the retention times of standard xyloglucan oligosaccharides.^{13,19-22)} The arrows in the figures indicate the elution positions of standard sugars, Glc, IP, FG (a), LG (b), LXG (c), LFG (d), LLG (e), XXXG (f), XXFG (g), XLFG and XLXG (h), XXLG (i) and XLLG (j).

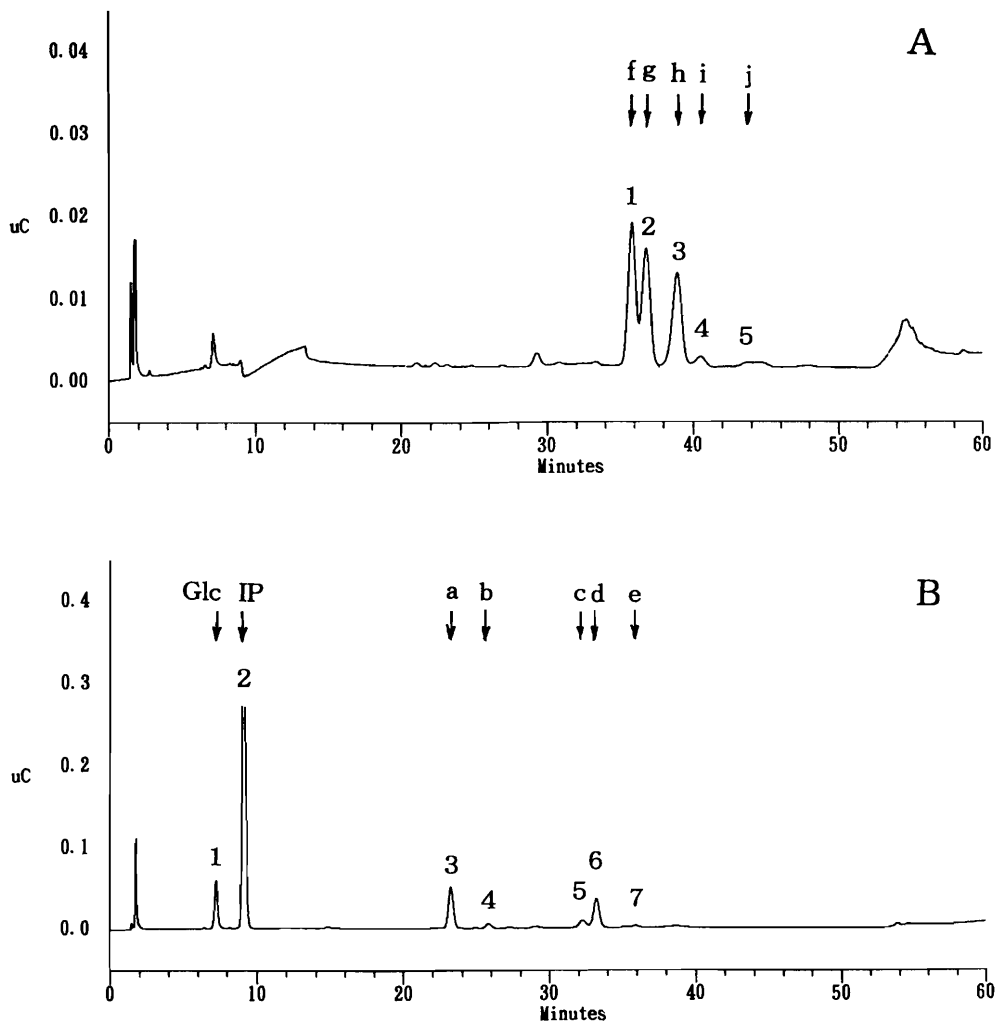


Table 2. Structural oligosaccharide units of xyloglucans obtained from the cell walls of cucumber, mung bean sprouts, and pumpkin.

Fruit vegetables	Oligosaccharide units (mol%)					
	XXXG	XXLG	XLXG	XXFG	XLLG	XLFG
Cucumber	36	4	6	31	1	22
Mung bean sprouts	37	8	5	30	1	19
Pumpkin	35	4	7	25	4	25

REFERENCES

- 1) Fry, S. C., York, W. S., Albersheim, P., Darvill, A., Hayashi, T., Joseleau, J. P., Kato, Y., Lorences, E. P., Maclachlan, G. A., McNeil, M., Mort, A. J., Reid, J. S. G., Seitz, H. U., Selvendran, R. R., Voragen, A. G. J., and White, A. R.: An unambiguous nomenclature for xyloglucan-derived oligosaccharides. *Physiol. Plant.*, **89**, 1-3 (1993).
- 2) Hayashi, T.: Xyloglucans in the primary cell wall. *Annu. Rev. Plant Physiol. Mol. Biol.*, **40**, 139-168 (1989).
- 3) Kato, Y. and Matsuda, K.: Examination of the fine structures of xyloglucans using endo-(1→4)- β -D-glucanases. In "Methods in Carbohydrate Chemistry, Vol.X", eds. BeMiller, J. N., Manners, D. J., Sturgeon, R. J. and Whistler, R. L., John Wiley & Sons, Inc., New York, p.207-216 (1994).
- 4) York, W. S., Darvill, A. G., and Albersheim, P.: Inhibition of 2,4-dichloro-phenoxyacetic acid-stimulated elongation of pea stem segments by a xyloglucan oligosaccharide. *Plant Physiol.*, **75**, 295-297 (1984).
- 5) McDougall, G. J. and Fry, S. C.: Structure-activity relationship for xyloglucan oligosaccharides with antiauxin activity. *Plant Physiol.*, **89**, 883-887 (1989).
- 6) Augur, C., Yu, L., Sakai, K., Ogawa, T., Sinay, P., Darvill, A. L., and Albersheim, P.: Further studies of the ability of xyloglucan oligosaccharides to inhibit auxin-stimulated growth. *Plant Physiol.*, **99**, 180-185 (1992).
- 7) Sone, Y., Makino, C. and Misaki, A.: Inhibitory effect of oligosaccharides derived from plant xyloglucan on intestinal glucose absorption in rat. *J. Nutr. Sci. Vitaminol.*, **38**, 391-395 (1992).
- 8) Yamatoya, K., Shirakawa, M., Kuwano, K., Suzuki, J. and Mitamura, T.: Effects of hydrolyzed xyloglucan on lipid metabolism in rats. *Food Hydrocolloids*, **10** 369-372 (1996).
- 9) Hensel, A.: Polysaccharides as cancer chemopreventive agents: Inhibition of the activity of direct acting nitroaromatic mutagens in *Salmonella typhimurium*. In *Abstract Book, XIX International Carbohydrate Symposium*, Abstract No. CP112, San Diego, U.S.A. (1998).
- 10) Konishi, T., Mitsuishi, Y. and Kato, Y.: Compositional analysis of the oligosaccharide units of xyloglucans isolated from the cell walls of leaf and root vegetables. *J. Appl. Glycosci.*, **45**, 401-405 (1998).
- 11) Kato, Y.: Carbohydrate composition of major fruit vegetables. *Bull. Fac. Educ., Hirosaki Univ.*, **78**, 99-107 (1997).
- 12) Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F.: Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, **28**, 350-356 (1956).
- 13) Kato, Y. and Matsuda, K.: Structure of oligosaccharides obtained by controlled degradation of mung bean xyloglucan with acid and *Aspergillus oryzae* enzyme preparation. *Agric. Biol. Chem.*, **44**, 1751-1758 (1980).
- 14) Bitter, T. and Muir, H. M.: A modified uronic acid carbazole reaction. *Anal. Biochem.*, **4**, 330-334 (1962).
- 15) Kato, Y. and Matsuda, K.: Distribution of xyloglucan in *Phaseolus aureus* seeds. *Plant Cell Physiol.*, **18**, 1089-1098 (1977).

- 16) Hakomori, S.: A rapid permethylation of glycolipid and polysaccharide catalyzed by methylsulfanyl carbanion in dimethyl sulfoxide. *J. Biochem.*, **55**, 205-208 (1964).
- 17) Lindberg, B.: Methylation analysis of polysaccharides. *Methods Enzymol.*, **28**, 178-195 (1972).
- 18) Mitsuishi, Y. and Kosugi, Y.: A novel enzyme hydrolyzing xyloglucan of *Penicillium* sp. M451. *Nippon Nogeikagaku Kaishi*, **67**, 581 (1993).
- 19) Mitsuishi, Y., Kato, T., and Kosugi, Y.: Characterization of isoprimeverose producing enzyme produced by fungal strain M9. In "Abstracts of Papers, Society for Fermentation and Biotechnology", Japan, Osaka, p.103 (1992).
- 20) Konishi, T., Mitsuishi, Y. and Kato, Y.: Analysis of the oligosaccharide units of xyloglucans by digestion with isoprimeverose-producing oligoxyloglucan hydrolase followed by anion-exchange chromatography. *Biosci. Biotechnol. Biochem.*, **62**, 2421-2424 (1998).
- 21) Kato, Y. and Matsuda, K.: Structure of oligosaccharides obtained by hydrolysis of mung bean xyloglucan with *Trichoderma viride* cellulase. *Agric. Biol. Chem.*, **44**, 1759-1766 (1980).
- 22) Matsushita, J., Kato, Y. and Matsuda, K.: Further structural study of an endo-cellulase-derived mung bean xyloglucan oligosaccharide, decasaccharide. *Agric. Biol. Chem.*, **49**, 1533-1534 (1985).
- 23) Kato, Y., Matsushita, J., Kubodera, T., and Matsuda, K.: A novel enzyme producing isoprimeverose from oligoxyloglucans of *Aspergillus oryzae*. *J. Biochem.*, **97**, 801-810 (1985).
- 24) York, W. S., van Halbeek, H., Darvill, A. G., and Albersheim, P.: Structural analysis of xyloglucan oligosaccharides by ¹H-NMR spectroscopy and fast-atom-bombardment Mass Spectrometry. *Carbohydr. Res.*, **200**, 9-31 (1990).

(1999.1.6 受理)