Bot. Bull. Academia Sinica **15**: 123-131 (1974)

A CHEMICAL STUDY OF THE CELL WALL POLYSACCHARISES OF CHINESE CELERY (*OENANTHE JAVANICA* DC)[1][2]

HSO-FRENG YUAN

Institute of Botany, Academia Sinica, Taipei, Republic of China

(Received for publication April 19, 1974)

Abstract

Chinese celery (*Oenanthe javanica* DC) is one of the fiber rich vegetables. The cell wall constitues 1.85% of its fresh weight. The cell wall preparations were extracted sequentially to obtain 34.22% pectic substances, 16.50% total hemicelluloses, 31.18% α-cellulose, 0.57% polysaccharide in delignification liquor, and 5.05% lignin. Each of these fractions, except for lignin, was hydrolyzed and the resultant sugars were isolated, identified, and estimated quantitatively.

Cell wall polysaccharides of chinese celery are made up of seven monoaccharides, i. e. glucose, galactose, xylose, arabinose, galacturonic acid, mannose and rhamnose. The main sugars in pectic substances are galacturonic acid, galactose and rhamnose. The component sugars of hemicelluloses are glucose, galactose, xylose, mannose and galacturonic aicd. According to the solubility of hemicelluloses in alkali, there are two types of hemicelluloses present in the cell wall of chinese celery. One is the xylose-rich polysaccharide which is easily extracted with 10% KOH, and the other is the mannose-containing polysaccharide which can be extracted with 24% KOH.

Introduction

The plant cell wall is a complex structure of polymers which is separated from the cytoplasm by the cell membrane. The cell wall counteracts the turgor pressure resulting from the cell contents and provides the rigid structure to hold the plant erect. The wall also plays a complex role in plant pathogenisis (Albersheim, 1969). It presents a physical barrier to the infecting pathogen. It can also be the nutrient for the pathogen. Moreover, it may control the production of degradative enzymes by the pathogen. Young cell walls are composed primarily of polysaccharides with a small amount of protein (Lamport, 1970), while mature cell walls, in addition to these compounds, contain lignin (Neish, 1965).

Increased attention is focused on the chemical compositions of plant cell

(1) This work was supported by the National Science Council, Republic of China.

(2) Paper No. 143 of the Scientific Journal Series, Institute of Botany, Academia Sinica.

walls with the intent to understand the relationship between the wall structure and metabolic functions. The chemical compositions of cell wall polysaccharide for plant verieties within a given species are essentially identical. However, differences in the sugar compositions were observed in cell walls prepared from the different species of the same as well as of different genera (Nevins, 1967). The hemicelluloses of the cell walls have been extensively studied in wood, fibrous plants and grains (Aspinal, 1959; King, 1963; Reid, 1969); but only to a limited extent in vegetables and fruits (Su, 1967; Knee, 1973).

The chinese celery (*Oenanthe javanica* DC) is one of the important vegetables in Taiwan. This paper reports the chemical compositions of the cell wall polysaccharides in the edible portion of chinese celery with emphasis on the carbohydrate-containing fractions; i. e. pectic substances, hemicelluloses, and α-cellulose.

Materials and Methods

Plant materials and general analysis

Fresh chinese celery (*Oenanthe javanica* DC) was perchased from the market near Taipei. After removing leaves and roots, the edible portion (stem) was washed with deionized water and blotted with tissue paper. General analysis was made according to the method described in the Methods of Analysis, AOAC (Horitz, 1960).

Preparation of cell wall

Cell wall was prepared according to the method of Hart and Kindel (1970). The edible portion of the fresh chniese celery (700 g) was choopped into small pieces, then homogenized for two minutes in a Waring blandor successively with cold 1 M-sodium chloride (three times), water (once), 0.1 M-sodium chloride (twice) and water (three times). All extraction operations were conducted in a cold room (4°C), and 2 litre quantities being used for each homogenization. The suspension was filtered through six layers of cheesecloth after each homogenization. The final residue was washed with water until the washings were free of Cl^-, then dried by solvent exchange with 95% ethanol, ethanol-diethyl ether (1:1 v/v), diethyl ether and desiccated to dry white material (13 g). This is designated as cell wall.

Extraction and fractionation of cell wall polysaccharides

1. Extraction of pectic substances: Pectic substances were extracted by a modified method of Dever (1968). Cell wall preparation (6.87 g) were extracted twice with one litre of ammonium oxalate-oxalic acid (0.5% each) at 90° for 24 hours. After each extraction, the residues were separated from the extracts by filtration through a sintered-glass funnel. The residues were washed with distilled water and dried by solvent exchange method as described in the last section. The two extracts were combined with the washings and

concentrated to less than 100 ml under reduced pressure in flash evaporator below 45°. The concentrated extracts was dialyzed against water for 24 hours at room temperature. To the dialysate, 8 volumes of 95% ethanol was added, slowly with stirring, and the mixture was allowed to stand at 4° overnight. The precipitated pectic substances (4.20 g) were collected by centrifugation, and dried by solvent exchange as described before.

2. Delignification of cell wall residue: The oxalate-extracted residue was delignified according to the method of Gaillard (1958). Water (300 ml) was added to the oxalate-extracted residue (3.49 g) to provide a slurry which was heated to 80° in a water bath. Chloramine-T (2 g to 75 ml of water) was added and followed by glacial acetic acid (1 ml to each 1 g of chloramine-T), and the mixture was kept at 80° for 2 hours with occasional stirring. The hot slurry was filtered through a sintered-glass funnel and the residue was washed with ethanol followed by boiling ethanolamine solution (3%, w/v, in ethanol) and finally ethanol. During the ethanolamine washing the residue was kept covered with the hot ethanolamine for 3 minutes before applying suction. The entire chloramine-T-ethanolamine treatment was repeated twice to give a final residue of hollocellulose (3.09 g) which was dried by solvent exchange method.

The delignification liquors which combined with the washings were concentrated to below 100 ml and filtered to remove some precipitates. The clear liquor was dialyzed against tap water for 72 hours. The polysaccharide (0.07 g) in the dialyzed liquor was precitated by adding 8 volumes of 95% ethanol, collected by centrifugation and dried by solvent exchange as described previously.

3. Extraction and fractionation of hemicelluloses and α-cellulose: The procedure of extraction and fractionation of hemicelluloses from the hollocellulose is outlined in Fig. 1. All alkaline extractions were carried out at room temperature by continuous stirring overnight under nitrogen and the extracts were filtered through a sintered-glass funnel. The residues were washed with 5% KOH followed by water and the washings added to the alkali extracts. The water-washed residues were further washed with 95% ethanol and dried by the solvent exchange method. The alkaline filtrates were acidified to pH 4.5 with acetic acid while cooled in ice bath. The hemicelluloses A_{10} and A_{24} precipitates were collected by centrifugation (20,000×g) after standing in ice bath for 2 hours. Hemicelluloses B_{10-s} and B_{24-s} were precipitated by adding 8 volumes of 95% ethanol to their solutions, slowly with stirring, and the mixtures were allowed to stand at 4° overnight. The resulting precipitates were collected by centrifugation and dried by the solvent exchange method.

Hydrolysis and estimation of monosaccharides

1. Hydrolysis of α-cellulose with H_2SO_4: α-Cellulose was hydrolyzed with

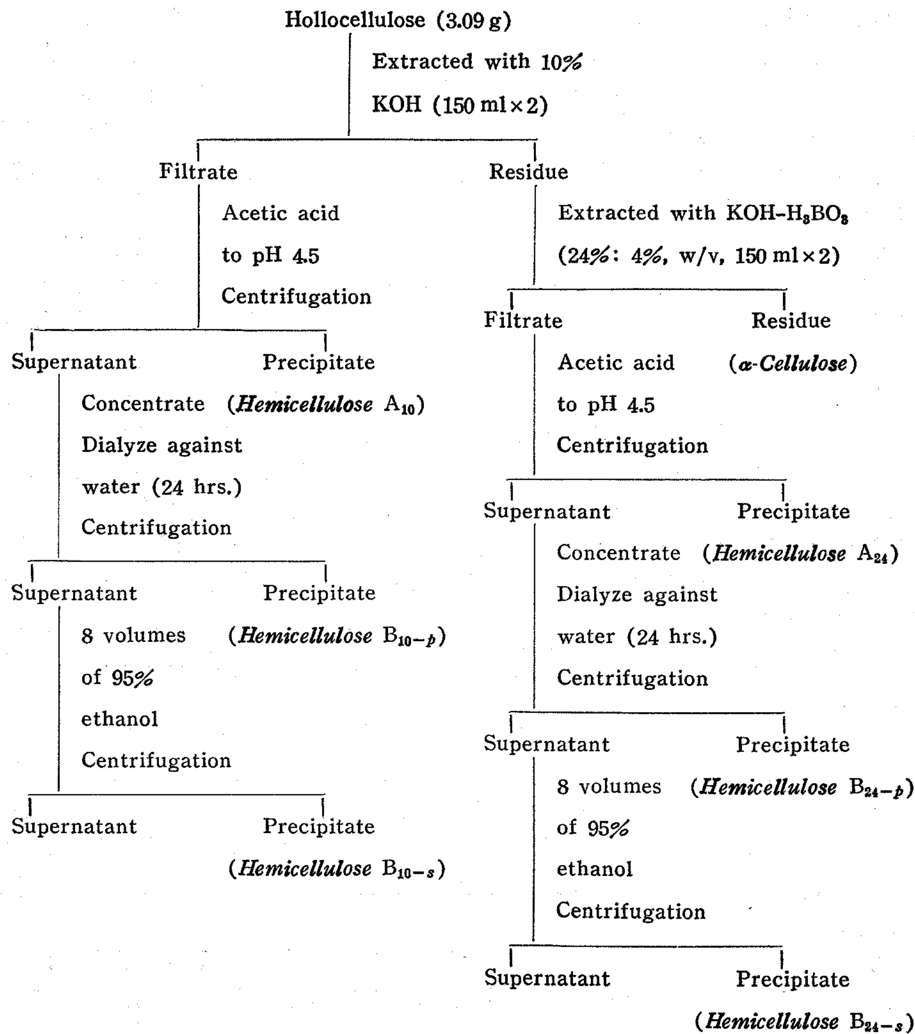

Fig. 1. Scheme for extraction and fractionation of plant hemicelluloses.

H_2SO_4 according to the method of Knee (1973). α-Cellulose (20 mg) was allowed to dissolve in 0.2 ml of 72% H_2SO_4 and stand at room temperature for 24 hours, then diluted with 2.5 ml of water. The solution was autoclaved at 121° for 60 minutes. The acid was neutralized by $BaCO_3$ and the white precipitate was removed by centrifugation. The resulting supernatant was concentrated for paper chromatography.

2. Hydrolysis of pectic substances and hemicelluloses with trifluoroacetic aicd: Pectic substances, polysaccharide in the delignification liquor, and hemicelluloses A_{10}, B_{10-p}, B_{10-s}, A_{24}, B_{24-p}, and B_{24-s} were hydrolyzed with trifluoroacetic acid by a modified method of Nevins (1967). Twenty mg of sample was weighed into a test tube (13×100 mm). Exactly 2 ml of 2N trifluoroacetic acid was introduced into the test tube. The tube was sealed and heated

at 121° for 60 minutes. The precipitates formed in the hydrolysates were removed by centrifugation. The supernatant fluid was transfered into a small beaker, and the precipitate was washed three times by re-suspansion in 0.5 ml portion of 70% ethanol followed by re-centrifugation. The supernatant fluid and the washings was combined and evaporated to dryness under an infra-red lamp and further dried in vacuum. Finally, the soluble sugars were dissolved in 1 ml of water for paper chromatography.

3. Paper chromatographic analysis of monosaccharides: Sugars in the hydrolysates were analyzed by decending paper chromatography. Three solvent systems, *n*-butanol-acetic acid-water (4:1:1 v/v), *n*-butanol-pyridine-water (6:4:3 v/v), and ethyl acetate-pyridine-water (8:2:1 v/v) were used. The identification of sugar spots was made by co-chromatography with authentic sugars. Sugars on the chromatograms were detected by using silver nitrate dipping method (Block *et al.* 1958) or by spraying with aniline hydrogen phthalate (Partridge, 1949).

4. Estimation of monosaccharides in the hydrolysates: The monosaccharide compositions in the hydrolysate were analyzed by a quantitative paper chromatographic technique as follows. The neutralized hydrolysate was applied on a large sheet of Whatman No. 1 filter paper as a streak with two guiding spots on both end. After developing the chromatogram at room temperature for 20 hours with a solvent system of ethyl acetate-pyridine water (8:2:1 v/v) by descending method, the separated sugars were located by the aid of guiding spots. The paper strips containing the sugars were cut out, eluted into a graduated test tube with water, and made up to 2 ml. The total sugar content in each tube was determined according to the method described in the Methods of Enzymology (Ashwell, 1957). Glucose, galactose, mannose and rhamnose were determined by cysteine–H_2SO_4 reaction; xylose and arabinose by orcinol reaction; and galacturonic acid by carbazole reaction.

Results

General analysis

The results of general analysis on the edible portion of the chinese celery are given in Table 1. The data are presented on dry matter basis except moisture. The protein content is calculated as 6.25 times the percentage of nitrogen. Depend upon these data, the nitrogen free extracts and cell wall polysaccharides in the dry matter of chinese celery can be estimated.

Compositions of cell wall

According to the data of cell wall preparation, the recovery of total cell wall is 43.29% in dry matter. Table 2 presents the percentage of fractionated cell wall polysaccharides based on the dry weight of the extracted fractions. The protein content in the cell wall is determined by Lawry method (1951).

Table 1. *General analvsis of edible portion of chinese celery*
Presented on dry metter basis except moisture

Compositions	Percentage (%)
Moisture	95.71
Protein	14.06
Fats	2.06
Fiber	31.22
Ash	8.70
Nitrogen free extracts*	43.96
70% Ethanol solubles	39.38
Cell wall polysaccharides**	35.80

* Nitrogen free extracts=Dry matter as 100%−Protein−Fats−Fiber−Ash
** Cell wall polysaccharides=Fiber+(Nitrogen free extracts−70% Ethanol solubles)

Table 2. *Compositions of cell wall of chinese celery in edible portion*
Presented on dry cell wall basis

Compositions	Percentage (%)
Pectic substances	34.22
Hemicelluloses:	
Hemicellulose A_{10}	3.17
Hemicellulose B_{10-p}	4.99
Hemicellulose B_{10-s}	5.66
Hemicellulose A_{24}	1.10
Hemicellulose B_{24-p}	0.19
Hemicellulose B_{24-s}	1.39
Polysaccharides in delignification liquor	0.57
α-Cellulose	31.18
Lignin-like substance	5.05
Protein	1.34
Total recovery	88.86

Analysis of monosaccharides of polysaccharide fractions

Paper chromatographic analysis showed that the component sugars of the cell wall polysaccharides are galacturonic acid, galactose, glucose, xylose, arabinose, mannose, and rhamnose. The pectic substances fraction contains galacturonic acid, galactose and rhamnose as its major component sugars as well as a small amount of glucose, arabinose and xylose. The polysacharide in delignification liquor has the same component sugars as in the pectic substances. In hemicelluloses, the component sugars are galacturonic acid, galactose, glucose, mannose and xylose. As for α-cellulose, the glucose should

be the only component sugar, however, there is still a very small amount of mannose that can be detected in its hydrolysate.

The component sugar ratios in the hydrolysate of fractionated polysaccharides have been estimated. The mole ratios of component sugars are shown in Table 3.

Table 3. *Mole ratios of component sugars in the fractionated polysaccharides*

Polysaccharide fractions	Monosaccharides						
	GalUA	Gal	Glu	Man	Ara	Xyl	Rha
Pectic substances	8.13	1.00	0.12	—	0.18	0.45	1.83
Polysaccharide in delignification liquor	2.60	1.00	0.12	—	0.15	2.28	1.91
Hemicellulose A_{10}	5.42	1.00	1.29	—	—	20.39	—
Hemicellulose B_{10-p}	—	1.00	4.24	—	—	41.45	—
Hemicellulose B_{10-s}	—	1.00	8.71	—	—	17.35	—
Hemicellulose A_{24}	52.66	1.00	8.06	5,04	—	5.43	—
Hemicellulose B_{24-p}	—	1.00	7.22	2.77	—	6.24	—
Hemicellulose B_{24-s}	—	1.00	3.48	3,74	—	1.61	—

Abbreviation used: GalUA=galacturonic acid; Gal=galactose; Glu=glucose; Man=mannose; Ara=arabinose; Xyl=xylose; Rha=rhamnose.

Discussion

Chinese celery is one of the fiber rich vegetables. Its cell wall preparation constitutes 1.85% of tissue fresh weight, and there are 34.22% pectic substances, 16.5% total hemicelluloses, 0.57% polysaccharide in delignification liquor and 31.18% α-cellulose (Table 2). There have been only a few reports of cell wall analyses. The difficulties in drowing comparisons between prior reports and the present data arise primarily from differences in the growth periods and the method of extracting verious fractions. Dever (1968) reported that cell wall of the mature corn root tissue was 1.03% of its fresh weight and that there are 15% pectin, 40% hemicelluloses and 24% α-cellulose. Odhnoff (1957) reported 7% pectin, 15% hemicelluloses and 21% α-cellulose in bean root cell wall. Cell wall analysis always involves a series of extractions in increasing severity of extracting solvents followed by detection and estimation of monomeric constituents in the extracted fractions. The ability of the extracting solvent to selectively extract wall components is certainly not perfect. The α-cellulose fraction serves as an example. This fraction still contains a small amount of mannose.

Pectic substances fraction contains a large amount of galacturonic acid, galactose and rhamnose as well as a small amount of glucose, xylose and arabinose. The presence of galactose and rhamnose in the pectic substances fraction shows that the galacturonic acid polymer is not the only component

to determine the wall rigidity. The polysaccharide in delignification liquor has the same component sugars as in the pectic substances, but with more xylose content (Table 3). Judging from the mole ratio of its component sugars, it seems that the polysaccharide in delignification liquor is mainly the pectic substances and contaminated with some hemicellulose.

It is apparent that there are two different types of hemicelluloses present in the cell wall of chinese celery (Table 3); the xylose-rich hemicelluloses which are easily extracted with 10% KOH, and the mannose-containing hemicelluloses which can be extracted with 24% KÓH. The hemicellulose fractions offers the most intriquing yet the most difficult feature of the cell wall to evaluate. Marked quantitative changes in the carbohydrate compositions are accompanied with the changes of solubility of various polysaccharide fractions. Glucose and xylose are the most abundant sugars in all hemicelluloses with regard to chemical compositions. The hemicelluloses in chinese celery are similar in carbohydrate compositions to other plants with exception that fucose and arabinose are not present; but a small amount of arabinose is found in pectic substances and polysaccharide in delignification liquor.

Literature cited

ALBRSHEIM, P., T. M. JONES, and P. D. ENGLISH. 1969. Biochemistry of the cell wall in relation to infective processes. Ann. Rev. Phytophathol. **7**: 171-194.

ASPINAL, G. O. 1959. Structural chemistry of the hemicelluloses. Advances in carbohydrate chemistry, Vol. **14**: 429-468, Academic Press, New York and London.

ASHWELL, G. 1957. Colorimetric anelysis of sugars. Methods in Enzymology, Vol. III. 73-105.

BLOCK, R. J., E. L. DURRUM, and G. ZWEIG. 1958 A manual of paper chromatography and paper electrophoresis. Academic Press. 178-180.

DEVER, J. E., R. S. BANDERSKI, and A. KIVILAAN. 1968. Partial chemical characterization of corn root cell walls. Plant Physiol. **43**: 50-56.

GAILLARD, B. D. E. and R. W. BAILEY. 1968. The distribution of galactose and mannose in the cell wall polysaccharides of red clover (trifolium patense) leaves and stems. Phytochemistry **7**: 2037-2044.

HART, D. A. and P. K. KINDEL. 1970. Isolation and partial characterization of apiogalacturonans from the cell wall of *Lemna minor*. Biochem. J. **116**: 569-579.

HORWITZ, W. 1960. Official Methods of Analysis of the association of official agricultural chemists. 9th. edition. 283-289, and 643.

KING, N. J. and S. T. BAYLEY. 1963. A chemical study of the cell walls of Jerusalem artichoke tuber tissus under different growth conditions. Can. J. Botany **41**: 1141-1153.

KNEE, M. 1973. Polysaccharides and glycoproteins of apple fruit cells. Phytochemistry **12**: 637-653.

LAMPORT, D. T. A. 1970. Cell wall metabolim. Ann. Rev. Plant Physiol. **21**: 235-270.

LOWRY, O. H., N. J. ROSEBRONGH, A. L. FARR, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**: 265-275.

NEISH, A. C. 1965. Coumarins, Phenylpropanes, and Lignin. In J. Bonner and J. E. Vanner, eds. Plant Biochemistry. Academic Press, Inc., New York. 581-614.

NEVINS, D. J., P. D. ENGLISH, and P. Albersheim. 1967. The specific nature of plant cell wall polysaccharides. Plant Physiol. **42**: 900-906.

ODHNOFF, C. 1957. Boron deficiency and growth. Physiol. Plantarum. **10**: 984-999.

PARTRIDGE, S. M. 1949. Aniline hydrogen phthalate as a spraying reagent for chromatography of sugars. Nature **164**: 443.

REID, J. S. G. and K. C. B. WILKIE. 1969. Total hemicellulose from oat plants at different stages of growth. Phytochemistry **8**: 2059-2065.

SU, J. C., D. S. TZOU, and H. H. TAI. 1967. Carbohydrate metabolism in the shoots of bamboo ***Leleba oldhami***. A structural study of cell wall polysaccharides. Bot. Bull. Acad. Sinica **8**: 339-352.

水芹細胞壁多醣類化學成份的研究

袁　　守　　方

中央研究院植物研究所

水芹是一種含纖維素很多的蔬菜，其細胞壁佔有其新鮮重量的1.85%。經精製後的細胞壁，用不同溶劑依序抽取其中的成份，可得34.2%果膠質，16.50%半纖維素，31.18%纖維素及5.05%木質素。以上各部份的抽出物，除木質素外，其他部份都用酸水解後，經分離，證明，定量後得知水芹細胞壁是由葡萄糖，半乳糖，木糖，阿剌伯糖，半乳糖醛酸，甘露糖及鼠李糖等七種單糖所構成，果膠質之主要成份為半乳糖醛酸，半乳糖及鼠李糖。半纖維素中之主要成份為葡萄糖，半乳糖，木糖，甘露糖及半乳糖醛酸。據分析結果顯示出水芹細胞壁中有二種類型的半纖維素；一種是富於木糖的多醣類，另一種是含有甘露糖的多醣類。