Journal of Korea TAPPI
Vol.37.No.5, 2005
Printed in Korea

Xyloglucan in the Differentiating Xylem of the *Populus deltoides* M.

Tae-Jin Eom† and Soung-Bae Park

(Received on September 10, 2005: Accepted on November 15, 2005)

ABSTRACT

The chemical composition of the differentiating xylem of *Populus deltoides* M. was investigated and compared with that of sapwood. The cell wall polysaccharides were extracted sequentially from adifferentiating xylem and fractionated with gel chromatography. The sugar composition of each fraction was analyzed with G..C and H.P.L.C.

The cell wall of the differentiating xylem is rich with the pectin substance and hemicellulose compared with that of sapwood. The water-extracted polysaccharides from the differentiating xylem were composed mainly of xylose and glucose residues. The sugar composition of some of the fractions in the gel filtration of purified H_2O polysaccharide suggest that xyloglucan was extracted with H_2O from differentiating xylem. Also, we can supposed that the purified H_2O polysaccharide might be xyloglucan from the spectrometric data(IR and NMR) of purified H_2O polysaccharide.

Keywords : xyloglucan, differentiating xylem, cell wall polysaccharide, gel chromatography, sugar composition.

1. Introduction

Plant tissue is mainly composed of the plant cell wall which is a non-living material. The plant cell wall provides the structural framework for the organisms and protects livingorganic materials from the external environmental stresses. Also, the plant cell wall is a very important resource as it found with as food, fiber and energy. The main chemical components of the woody plant cell wall are polysaccharides (cellulose and hemicellulose) and the phenolic compound (lignin).

Since it is known that the polysaccharides of high plants are bioactive regarding the development and growth of plant cells, Sakurai et al (1) suggested that plant cell wall components control the elongationand development of cells by itself. For example, xyloglucan

• Department of Wood Science and Technology, College of Agriculture and Life Sciences, Kyungpook National University, 1370 Sangyeok-dong, Buk-gu, Daegu 702-701, Korea
† Corresponding Author : E-mail ; tjeom@knu.ac.kr

could promote the elongation of pea stems (2). It was reported that the polysaccharides of a plant cell wall controlled the expansion of that cell wall and the hardening of the pressure-induced cell wall (3,4).

The structure, the biosynthetic mechanism and function of the cell wall, is a very important aspect in understanding the plant kingdom. A clearer chemical understanding of the wood cell wall should be valuable to the wood and paper industries. The role of pulp hemicellulose in the hornification of kraft pulp was investigated by Oksanen et al (5)and the physical properties of recycled pulp was evaluated by Wistara and Young (6). They concluded that the changes in the physical properties of pulp in the paper making process is mainly due to the quantitative and qualitative changes of hemicellulose in pulp.

In general, it is difficult to study the chemical characteristics of polysaccharides in the cell wall of woody plants because of the pure isolation of raw materials without any chemical modification from deeply lignified wood tissue was very difficult. In particular, it was almost impossible to directly isolate polysaccharides from the primary cell wall. Therefore, most studies regarding the chemical characteristics of wood cell wall polysaccharides have been concerned with raw materials for experimentation. Sultze (7)isolated the extremely young tissues, which did not have any lignin nor cell wall thickening from an aspen-wood, and discussedits chemical composition. The results indicated that the extremely young tissues were very rich in pectin substance and very poor in glucan. Simson and Timell (8-12) discussed the fractionation and characterization of various hemicelluloses (xyloglucan, arabinogalactan and xylan) and pectin in the alkaline extract from cambial tissue.

On the other hand, Thomas et al (13) discussed the chemical composition of the primary cell wall in suspension cultured cell of Douglas fir and concluded that the cell walls were more similar to the dicot (sycamore) cell walls than to those of the graminaceous monocots because they had a predominance of xyloglucan over xylan as the principle hemicellulose. It can be believed that the cell wall polysaccharides could be prepared without any chemical modification from the differentiating xylem which lignification of the secondary wall has not started noticeably. Because a very low lignin content, the polysaccharides can be extracted from the cell wall without any delignification procedures that would lead to the chemical modification of polysaccharides.

In this paper, the cell wall polysaccharides were extracted sequentially from the differentiating xylem which was reflected very early stages of the lignification of aspen-wood(*Populus deltoides*. M) and were fractionated with gel-chromatography.

The purified H_2O extracted polysaccharide was analyzed with spectrometric instruments (IR and NMR) and the characteristics of the polysaccharides in the differentiating xylem were discussed.

2. Materials and Methods

2.1 Preparation of differentiating xylem (DX)

Twenty-years old aspen-wood(*Populus deltoides* M.) was cut in early June, 2001 in Chungsong (the university forest), Kyungsangbukdo, Korea. After peeling off the bark, the DX was collected carefully by scratching the surface with a dull steel blade.

The DX was kept in 90% ethanol and was heated at 70℃ for 2 hr. in order to deactivate enzymes. After powdered and/or colloidal contaminants were removed from the air dried samples, fibrous DX was powdered with an Willy mill to pass through a 40 mesh screen. The DX was extracted with a methanol/chloroform (1:1) solution under N_2 gas for 6 hr. to remove any fatty substances. After drying, the DX was extracted with mixed solution of phenol/acetic acid/water (2:1:1) for 6 hr. at room temperature to remove crude protein. The sapwood was also powdered with a Willy mill and a 20-80 mesh wood meal was extracted with mixture of methanol/chloroform(1:1) solution and dried (14).

2.2 Chemical composition

After being hydrolyzed with 3% H_2SO_4 of the DX, wood meal and the polysaccharides, the hydrolyzed solution was set at a pH of 5.5 with $Ba(OH)_2$.

$BaSO_4$was removed by centrifugation. After the sugar solution was reduced with sodium brohydrate, the alditol acetates or alditols of the neutral sugars was analyzed with G.L.C and H.P.L.C.. The conditions of G.L.C and H.P.L.C. are same as the previous report (14).

The acidic sugar content was determined by the modified Blumenkrantz method (15).

2.3 Spectromeric analysis

The IR spectra of the purified polysaccharides were measured on a IFS 120 HR (Bluker Co.) with KBr method. ^{13}C-NMR spectra of the purified polysaccharides were measured in D_2O(Aldrich chemical Co.) with a AM-500 spectrometer(Bruker).

2.4 Extraction of polysaccharides and gel chromatography

The DX (20g) was extracted with 200ml of H_2O, 0.05M of Na_2CO_3, 1M of KOH, and 4M of KOH and 4M of KOH+ Boric acid(1:1) solution for 3 hr at room temperature sequentially (14)The extracts were neutralized immediately with acetic acid and dialyzed with a cellulose membrane. The polysaccharides were concentrated with a rotary evaporator and lyophilizated.

The lyophilizated polysaccharides (200mg) were dissolved in 100ml of 2N NaOH and filtered by glass microfibre filter to remove nonsoluble compounds.

The filterates were acidified immediately to a pH of 4 with aceticacid and were kept in a refrigerator overnight. The precipitates were recovered by centrifugation and were washed with water, ethanol and ether in that sequence.

The polysaccharides were dried at 60℃ with a vacuum and the sugar composition was analyzed with G.L.C.

The purified polysaccharides (50mg) were solved in 1N of NaOH and were charged in a column(1001.5cm) of Bio-gel P-2 and eluted with 0.1N NaOH. Every 5ml of elute were fractionated in a test tube. The reducing sugar content in every fraction (0.5ml) was determined by the phenol-H_2SO_4 method.

The fractions (4.5ml) of the high absorbance (485nm) were neutralized with 0.1N of acetic acid and mass up to 10ml of 3% H_2SO_4 with water and cons. H_2SO_4. The fractions were hydrolyzed in an autoclave at 120℃ for 1.5 hr. The neutral sugars were reduced with $NaBH_4$ and analyzed with H.P.L.C.

3. Results and Discussion

3.1 Chemical composition of the DX and sapwood.

Table 1. Elementary composition and lignin content of DX and sapwood(%)

	Carbon	Hydrogen	Oxygen	Nitrogen	Lignin		
					Total	Klason	UV
DX	40.9	6.5	52.6	1.1	6.2	1.8	4.4
Sapwood	46.6	6.9	46.5	0.0	23.8	20.1	3.7

The DX should have a high amount of protein which is mainly originated from cytoplasmic materials and enzymes bound to the cell wall. These proteins should be removed from the cell wall materials because it disturbs the extraction of polysaccharides and the chemical analysis of the chemical composition (16).

Table 1 shows that the element compositionand lignin content of the DX that were deproteinized with extraction of a mixed solution(pH-OH/ Ac-OH/H_2O : 2/1/1) and sapwood.

As the DX has more oxygen and lower carbon than sapwood, the level of acidic polysaccharides, such as pectin, might be higher in the DX than sapwood. The small amount of protein which has a very high chemical affinity to the cell wall component could not be removed sufficiently from the DX.

The lignin content in the DX was 6.2% which is 1.8% of the Klason lignin and 4.4% of the UVlignin. Since the proteins in the plant cell wall have a very high affinity to lignin, it can be supposed that some parts of the Klason lignin in the DX are proteins. The amount of proteins, however, are negligible for further procedures of polysaccharide extraction and purification.

Therefore, the DX is composed of the cell wall at a very early stage of lignification. The polysaccharides in the DX should be extracted easily using alkaline solutions without any regarding deligninfication.

3.2 The sugar composition of the DX and extracted polysaccharides

The sugar composition of the DX was compared with that of sapwood in Table 2. The DX contains a much higher content of acidic sugar than sapwood. It can be agreed that the acidic sugar may have originated from the pectin substance which contains a high amount of glucuronic and/or galacturonic acid. The pectin substance can adhered itself to a cell wall lignin in the middle lamella (12, 17). Even though not all glucose residues come from cellulose, the low content of glucose in the DX means that the deposition of cell wall cellulose is not sufficient but non-cellulosic polysaccharides are deposited fully. The high content of arabinose and xylose residues means that there is a high amount of arabinogalactan and xylan in the DX. The xylose and glucose residues might be from the xyloglucan in the

Table 2. Sugar composition of DX and sapwood(%)

	Total neutral sugar	Relative neutral sugar component					Acidic Sugar
		Ara	Xyl	Man	Gal	Glu	
DX	48.5	10.7	24.7	2.7	7.0	52.9	35.6
Sapwood	63.4	3.6	22.9	3.2	2.8	67.5	2.3

Table 3. Sugar composition of extracted polysaccharides(%)

Extract	Yield	Relative neutral sugar composition					Acidic Sugar
		Ara	Xyl	Man	Gal	Glu	
H_2O	8.5	5.0	22.6	1.8	5.4	63.2	20.5
Na_2CO_3	21.3	22.0	26.2	19.0	23.0	9.8	45.3
1M KOH	6.0	20.5	58.7	8.5	8.9	2.4	3.5
4M KOH	7.8	9.9	65.0	1.5	5.6	18.0	1.2
" $+H_3BO_3$	8.2	22.8	8.0	1.7	67.4	-	0.8
Residu	41.4	5.0	5.4	2.3	2.4	84.9	-

DX which is isolated from the cambial tissue of the *Populus tremuloides* and *Tilia americana* by Simson et. al. (11) and isolated from suspension cultured cells by Thomas et. al. (13).

The sugar composition and yield of the polysaccharides extracted in sequence from the DX is shown in Table 3.

The yield of the water extraction polysaccharide is 7.5% and is composed mainly of xylose, glucose residues and acidic sugar moiety. It might be supposed that the xylan and glucan and/or xyloglucan and pectin substances were extracted with water from the DX.

The Na_2CO_3 extract is more than 20% and most of the pectin substances and various polysaccharides were extracted with Na_2CO_3.

The 1M KOH extract was composed with arabinose and mainly xylose residues which may have originated from xylan. The 4M KOH extract was composed of mainly xylose and glucose residues. It can be supposed that some of xyloglucan in the DX was extracted with the 4M of KOH. The arabinogalactan was extracted with 4M of KOH+H_3BO_3. The residue was composed of glucose residues of cellulose.

3.3 Sugar composition and IR spectra of purified polysaccharides

It might be very difficult to isolate xyloglucan from mature wood because xyloglucan is well attached to the cellulose microfible near the primary cell wall by a hydrogen bond. Also, the relative amount of xyloglucan in mature xylem is very small. A relatively high amount of xyloglucan, however, should be isolated from the DX where the deposition of other cell wall components was not sufficient. It can be expected that xyloglucan can be extracted by some solution in Table 4.

The polysaccharides that were extracted with water and 4M of KOH, which are expected to contain xyloglucan moity and also 4M of KOH+H_3BO_3 polysaccharides, were further purified and the sugar compositions of purified

Table 4. Yields and relative neutral sugar composition of purified polysaccharides(%)

Polysacchride	Yield	Ara	Xyl	Man	Gal	Glu
H_2O	50.5	1.1	22.0	-	3.2	70.7
4M KOH	72.2	4.5	69.4	-	-	26.0
" $+H_3BO_3$	74.0	32.3	3.2	-	64.6	

polysaccharides were analyzed.

As shown in Table 4, the purified H_2O extracted polysaccharide was composed of mainly xylose and glucose residues. It can be supposed that a kind of xyloglucan in the DX was extracted with water.

The purified 4M of KOH extracted polysaccharides(72.2% of yield) were composed also of mainly xylose and glucose residues. The ratio of xylose and glucose residues, however, was the opposite of the H_2O extract. This means that the purified 4M KOH extracted polysaccharides were a mixture of main xylan and glucan or xyloglucan.

The purified 4M KOH+H_3BO_3 polysaccharides show a high yield and were composed of mainly arabinose and galactose residues which originated from the arabinogalactan.

The IR spectra of purified polysaccharides were shown in Fig. 1.

Makurakova et al. (18) tried to identify the particular polysaccharides with infrared spectra by a FT-IR spectrometer and have concluded that the main IR band positions were influenced by the relative position of the axial and equatorial (OH) groups on the pyranoid ring. The IR spectra in 1200-800 cm^{-1} region provide information about the main polysaccharides present in the complex systems of polysaccharide mixtures.

They concluded that the strong peak of around 1040 cm^{-1} of the purified H_2O extracted polysaccharide might have originated from xyloglucan or β-glucan. It can be expected that the purified H_2O extracted polysaccharides contained xyloglucan and/or β-glucan. The FT-IR spectrum in the 1200-800 cm^{-1} region of the purified H_2O extracted polysaccharide was quite different from that of the purified 4M of KOH polysaccharide which is supposed to a mixture of xylan and glucan.

3.4 Gel chromatography and sugar composition of fractions

The purified water, 4M of KOH and 4M of KOH+H_3BO_3 extracts polysaccharides, were fractionated with Bio-gel P2 for further investigation of the chemical characteristics.

The gel chromatography of the purified water extract polysaccharides on the Bio-gel P2 are shown in Fig. 2 and the sugar composition of some fractions in the gel chromatography are in Table 5.

The gel chromatogram shows one main peak containing 5 fractions.

Each fraction was composed of xylose (about 30%), galactose (3%) and glucose (65%)

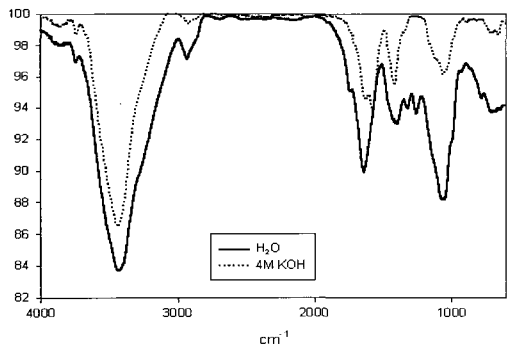

Fig. 1. IR spectra of purified polysaccharide.

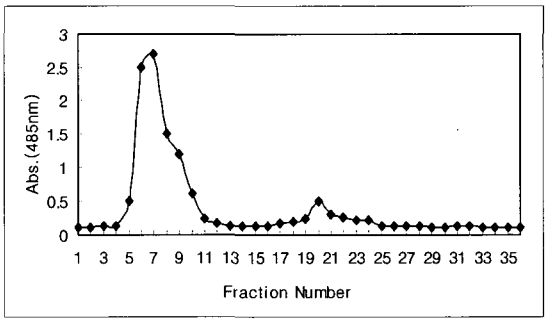

Fig. 2. The gel chromatography of purified water extract polysaccharides on Bio-gel P2.

Table 5. Relative neutral sugar composition(%) of fractions in Fig. 2

Fraction No. in Fig. 2.	Ara.	Xyl.	Man.	Gal.	Glu.
6	-	30.5	-	3.3	66.2
7	-	29.2	-	3.5	67.3
8	-	30.9	-	4.0	65.1
9	-	28.7	-	3.9	67.4
10	-	31.7	-	3.8	68.5

residues. If the purified water extract polysaccharides were a mixture of xylan and some glucan, the gel chromatogram could have two or more main peaks and the sugar composition of each fraction would changed greatly. The constant sugar composition of each fraction means that this purified polysaccharide is not a mixture but a homogeneous substance. Therefore, it can be suspected that this purified polysaccharide is xyloglucan. Albersheim and his coworkers (19, 20) suggested that xyloglucan is attached to cellulose in primary cell wall by strong hydrogen bonds. Also, it is known that xyloglucan does not occur in the secondary wall and seems to be restricted to the primary walls. That is why it is difficult to isolate xyloglucan from mature wood tissue. The xyloglucan can be extracted from differentiating xylem where lignification has not yet started. It is, however, very unusual that water extract from the DX contains polysaccharides such as xyloglucan because water soluble polysaccharides should have the weakest linkage to the cell wall complex. In order to understand water soluble xyloglucan, further investigation is needed.

The approximate ratio of xylose/glucose (0.46) in fractions from the gel chromatography of water extract polysaccharides is lower than that of xylogrucans in the alkaline extract from the cambial tissue of the *Populus tremuloides* (0.7) and *Tilia americana* (0.65) in Simson and Timell's report (9). The fucose residue was not detected from this water extract polysaccharide fraction. This also, did not correspond with the results of Simson and Timell (9). The xyloglucan in the water extract polysaccharides from the DX contained residues of xylose and glucose in approximate molar ratio of 1 : 2 with a small amount of galactose residue.

The gel chromatography of the purified 4M of KOH extract polysaccharides on the Bio-gel P2 are shown in Fig. 3. The sugar composition

Table 6. Relative neutral sugar composition(%) of fractions in Fig. 3

Fraction No. in Fig. 3.	Ara.	Xyl.	Man.	Gal.	Glu.
12	3.8	95.0	-	1.2	-
13	2.8	94.5	-	2.7	-
14	3.2	94.8	-	2.0	-
15	4.0	94.1	-	1.9	-
20	2.2	1.0	-	1.3	96.5
21	2.2	-	-	0.8	97.0

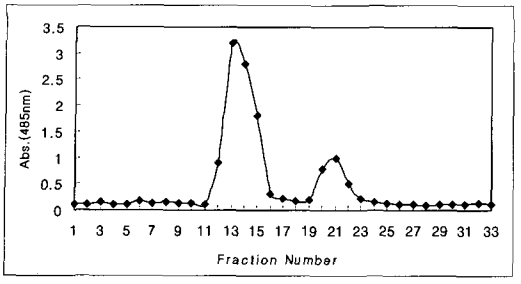

Fig. 3. The gel chromatography of purified 4M KOH extract polysaccharides on Bio-gel P2.

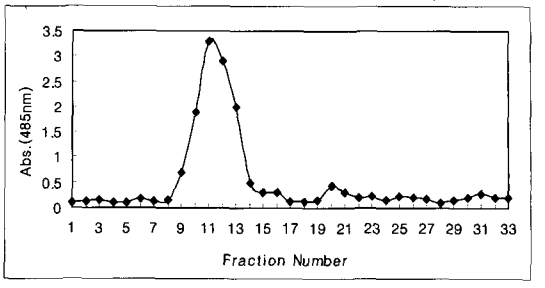

Fig. 4. The gel chromatography of purified 4M KOH+H_3BO_3 extract polysaccharides on Bio-gel P2.

of some fractions in the gel chromatography are in Table 6.

The gel chromatogram of the purified 4M KOH polysaccharides shows two main peaks containing 4 and 2 fractions. As a result of sugar composition analysis in each fraction, it is proven that the purified 4M KOH polysaccharides contain mainly xylan and a small amount of low molecular weight glucan.

Therefore, it can be concluded that the glucose residue of purified 4M KOH polysaccharides in Table 5 originated from glucan but not from xyloglucan. The existence of glucan in purified 4M KOH polysaccharide can be explained by the origin of the absorbance spectrum at 1040 cm^{-1} in Fig. 1.

The gel chromatography of purified 4M KOH+H_3BO_3 extract polysaccharides on Bio-gel P2 is shown in Fig. 4. The sugar composition of some fractions in the gel chromatography are in Table 7.

The gel chromatogram of purified 4M KOH+H_3BO_3 extract polysaccharides shows one main peak containing 4 fractions. From the sugar composition of each fraction, it is known that arabinogalactan was extracted from the DX by the 4M KOH+H_3BO_3 solution. The arabinogalactan from the DX has residues of arabinose and galactose in a molar ratio of about 1 : 2. These results are in agreement with those of Simson and Timell (10).

In comparing gel chromatography, the molecular weight of xyloglucan is higher than that of xylan and arabinogalactan.

The various polysaccharides were extracted from the DX with alkaline solutions in sequence. The polysaccharides were weakly attached to cell wall matrix and located on the near surface of the cell wall. They were extracted more easily by a weak alkaline solution. Since the xyloglucan is known to have a strong hydrogen bond to cellulose microfibrils

Table 7. Relative neutral sugar composition(%) of fractions in Fig. 4

Fraction No. in Fig. 4.	Ara.	Xyl.	Man.	Gal.	Glu.
10	33.8	2.1	-	64.1	-
11	32.6	1.0	-	66.4	-
12	33.2	1.8	-	65.0	-
13	34.4	-	-	65.6	-

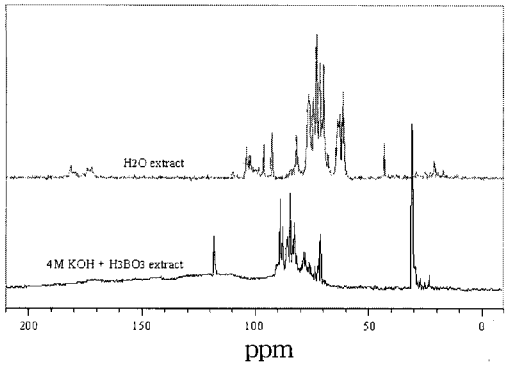

Fig. 5. C^{13}-NMR Spectra of Purified Polysaccharides.

in the primary cell wall, it can be agreed that xyloglucan in water extract polysaccharide wasn't the hydrogen bond to the cellulose microfibrils at this point in time.

C^{13}-NMR spectra of purified polysaccharides showed in Fig. 5.

As the case of IR spectra, chemical shift of carbon atoms of the purified H_2O extracted polysaccharides in ^{13}C-NMR spectra are quite different with that of purified 4M KOH+H_3BO_3 extract polysaccharides that are composed with mainly arabinogalactan. Especially, the strong signals near the 60 ppm of the purified H_2O extracted polysaccharide might origin from the C6 of glucose moieties in the xyloglucan.

4. Conclusion

The chemical composition of the differentiating xylem of *Populus deltoides* M. was investigated and compared with that of sapwood. The cell wall polysaccharides were extracted sequentially from a differentiating xylem and fractionated with gel chromatography. The sugar composition of each fraction was analyzed with G..C and H.P.L.C.

The cell wallof the differentiating xylem is rich with the pectin substance and hemicellulose compared with that of sapwood. The water-extracted polysaccharides from the differentiating xylem were composed mainly of xylose and glucose residues. The sugar composition of some of the fractions in the gel filtration of purified H_2O polysaccharide suggests that xyloglucan was extracted with H_2O from differentiating xylem. Also, we can supposed that the purified H_2O polysaccharide might be xyloglucan from the spectrometric data(IR and NMR) of purified H_2O polysaccharide.

Literature Cited

1. Sakurai, N., Yamamoto, R., Katou, Y., Cell wall of plant and polysaccharides, Baihukan, Tokyo, pp 32-47 (1991).
2. Shimizu, Y., Hayashi, T., Kawada, T., Sakuno, T., Promotion of pea stem elongation by the fragments of plant cell wall polysaccharides, Mokuzai Gakkaishi 43(2): 121-127 (1997).
3. Whitney, Sarah E. C., Gidley, Mike J., McQueen-Mason, Simon J., Plant Journal 22(4): 327-334 (2000).
4. Kasai, M., Nakagawa, A., Hatae, K., Shimada, A., Role of cell wall components in the hardening of pressure-treated Japanese radish, Nippon Kasei Gakkaishi 50(3): 251-257 (1999).
5. Okasnen, T., Buchert, J., Vikarri, L., The role of hemicelluloses in thehonification of bleached kraft pulps, Biotechnol. Food Res. 35(2): 134-141 (1997).
6. Wistara, Nyoman and Young Raymond A., Phygycal and chemical properties of pulps, Cellulose 6(4): 291-324 (1999).
7. Rolland F. Sultze, JR, A study of the developing tissues of aspenwood, Tappi 40(12): 985-995 (1957).
8. Simson, B.W. and Timell, T.E., Polisaccharides in cambial tissues of Populus tremuloides and Tilia americana, Cellulose Chem. and technol. 12: 39-50 (1978).
9. Simson, B.W. and Timell, T.E., Polisaccharides in cambial tissues of Populus tremuloides and Tilia

americana, Cellulose Chem. and technol. 12: 51-62 (1978).
10. Simson, B.W. and Timell, T.E., Polisaccharides in cambial tissues of Populus tremuloides and Tilia americana, Cellulose Chem. and technol. 12: 63-77 (1978).
11. Simson, B.W. and Timell, T.E., Polisaccharides in cambial tissues of Populus tremuloides and Tilia americana, Cellulose Chem. and technol. 12: 79-84 (1978).
12. Simson, B.W. and Timell, T.E., Polisaccharides in cambial tissues of Populus tremuloides and Tilia americana, Cellulose Chem. and technol., 12: 137-141 (1978).
13. Thomas J.R, McNeil, M., Darvill, A.G., Albersheim, P., Structure of Plant Cell Wall, Plant physiol. 83: 659-671 (1987).
14. Eom, T.-J., Park, Y.-J., Chemical characteristics of Cell-Wall polysaccharieds in the differentiating xylem of Populus deltoides M., Mokchae Konghak 26(3): 26-32 (1998).
15. Blumenkrantz, N. and Gustav, A.-H., New method for quantiative determination of uronic acid, Analytical biochemistry 54: 484-489 (1973).
16. Eom, T.-J., Meshisuka, G., Nakano, Z., Chemical characteristicsof lignin in the differentiating xylem of a hardwood, Mokuzai gakkaishi 33(7): 576-581 (1987).
17. Edashige, Y., Ishii, T., Hiroi, T., Structure analysis of polysaccharides of primary walls from xylem differentiating zones of crytomeria japonica, Holzforshung 49: 197-202 (1995).
18. Macuraková, M., P. Capek, V. Sasinkova, N. Wellner, A. Ebringerova, FT-IR study of cell wall model compounds: pectic polysaccharides and hemicelluloses, Carbohydrate Polymers 43: 195-203 (2002).
19. Albersheim, P., Talmadge, KW., Keegstra, K., Bauer, WD The structure of plant cell walls(1), Plant Physiol., 51: 158-178 (1973).
20. Albersheim, P., Darvill, A. G., Lau, K. R., Mcneil, J. M., Sharp, M., York, J.K., Structure, function and biosynthesis of plant cell walls, American society of-plant physiologists, USA, PP.19-51 (1984). --65.6 ------34.4137.00.8------2.221