

Film formation and some structural features of hemicellulose fractions from *Pinus densiflora* leaves

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Abstract Biodegradable translucent and transparent films were prepared from the hemicellulose fractions of *Pinus densiflora* leaves without using substantial amounts of additives. These fractions were isolated by alkaline extraction using 10 % sodium hydroxide after delignification by sodium chlorite, and precipitated with 80 % ethanol. The water-soluble fraction of the *P. densiflora* hemicellulose was mainly composed of arabino-4-*O*-methylglucuronoxylan according to a sugar analysis and NMR spectroscopy. The water-insoluble fraction contained more mannan-related polysaccharide than water-soluble fraction. Whole hemicellulose and the water-insoluble fraction formed stable films when they were dispersed in water, cast into petri dishes without additives, and dried. The water-soluble fraction formed the most transparent film, and it required a 1 % (w/w of polysaccharide) lecithin additive, while it was peeling off from the dish to be used as a self-supporting film. The 10–12 μm thick films had an average tensile strength of 36–43 MPa, and 2.1–2.5 % elongation at break. In the long wavelength, the light transmission was high in the order of water-soluble, whole hemicellulose, and water-insoluble fraction. *P. densiflora* leaves have a history of being edible; thus, the leaves of this tree can produce a useful and edible hemicellulose film.

Keywords *Pinus densiflora* · Leaf · Hemicellulose · Film

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Introduction

The needle-like leaves of *Pinus densiflora* (Japanese red pine, akamatsu) have been used since a long time for preparing folk medicine. These leaves have been historically used as a crude plant drug dating back to the Edo era in Japan, and its description can even be found from earlier ages in China [1]. The dried powder of whole *P. densiflora* leaves is commercially available as tea. There are many reports on the ingredients [1–3] and biological functions [4, 5] of the essential oil and volatile fraction of *P. densiflora* leaves; however, only limited information is available on its hemicellulose component.

Recently, biodegradable and/or edible films produced from plant-based polysaccharides such as hemicelluloses and celluloses have attracted attention as replacements for petroleum-based films. According to a report by Mikkonen and Tenkanen [6], xylans and mannans obtained from agriculture and forest industries could be a source of sustainable packaging materials in the future. The properties of xylan- and mannan-based films are suitable for food-packaging, although in some cases these films require components such as plasticizers to maintain self-supporting forms [7–9]. The property of produced films from hemicellulose has been studied for decades, and their production from the wood meal of *P. densiflora* has been previously reported from the standpoint of sorption of water vapor [10].

Some edible films produced from polysaccharides are widely used in the food industry, for example, oblate, pullulan, and agar. Films from hemicelluloses may also be used in the food industry. Although they are not very common as edible material, *P. densiflora* leaves are a sustainable bioresource that can be obtained from established forests. To facilitate the future utilization of

hemicellulose-based films derived from these leaves as edible films, we report some of their structural characteristics and mechanical properties.

Materials and methods

Materials and reagents

Dry powder of *P. densiflora* leaves for a beverage was purchased. It was prepared from *P. densiflora* leaves collected domestically and was washed, dried, and pulverized. Beech xylan and birch xylan were obtained from Sigma and Fluka, respectively. Dextran standards with molecular weights of 1000–410000 were purchased from Sigma. The dextran standard with a molecular weight of 668000 was obtained from polymer standards service. Lecithin from soybean was obtained from Wako, and 4-*O*-methylglucuronic acid (MeGlcUA) prepared from beech xylan was provided by Dr. S. Yoshida (University of Tsukuba); a single spot on TLC analysis verified its purity.

General analyses

To determine the acid-insoluble lignin content of *P. densiflora* leaves, the powder was boiled in an 80 % ethanol solution for 10 min, collected on a G3 fritted glass filter, and air-dried before acid hydrolysis of the powder using dilute sulfuric acid [11]. The ash content was determined and subtracted from the apparent acid-insoluble material to calculate the acid-insoluble lignin content. The relative sugar contents of polysaccharides were analyzed after hydrolysis of polysaccharides using 2 M TFA, then trimethylsilyl methyl ester glycoside derivatives were detected by GC-14A gas-liquid chromatography (GLC) (Shimadzu) equipped with a DB-1 column (0.25 mm × 30 m, J & W Scientific) [12]. The following seven neutral sugars were analyzed as compositional sugars: arabinose (Ara), fucose (Fuc), rhamnose (Rha), xylose (Xyl), mannose (Man), galactose (Gal), and glucose (Glc); in addition to the following three kinds of acidic oligosaccharides: glucuronic acid (GlcUA), galacturonic acid (GalUA), and MeGlcUA. Methylation analysis of polysaccharides was conducted according to the modified method of Hakomori [13, 14]. The partially methylated alditol acetates were analyzed by GLC-MS (JEOL AMIII15) using an SP-2330 column (0.25 mm × 30 m, Supelco). ¹³C-NMR spectra were recorded at 49.5 °C in D₂O on a JEOL α500 spectrometer operating at 125.65 MHz. Chemical shifts (ppm) were measured relative to the internal dioxane at 67.40 ppm or internal trimethylsilylpropionic acid (TSP) at 0.00 ppm. The particle size distribution was measured using a laser diffraction

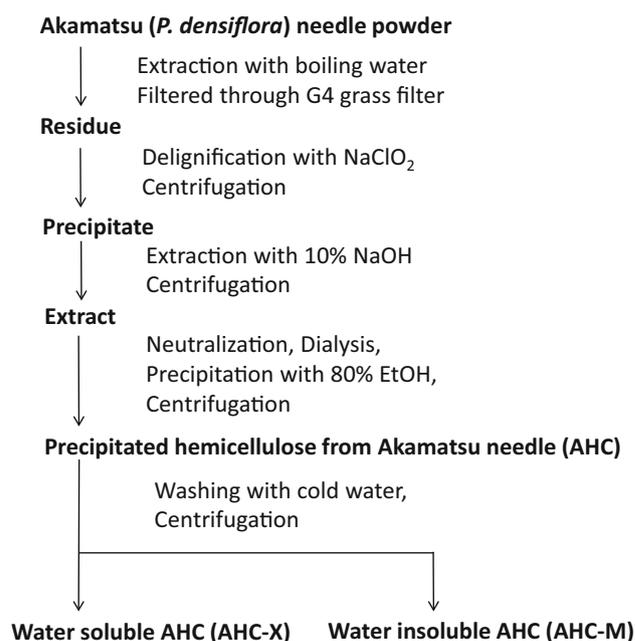


Fig. 1 Preparation of hemicellulose fractions from *Pinus densiflora* leaves

spectrometer HELOS/KR along with a dry disperser RODOS/L (HELOS & RODOS) at the Japan Laser Corporation (Tokyo, Japan).

Hemicellulose preparation

The hemicellulose fraction was prepared using 120 g (dry material (DM), 115.4 g) of the powder from *P. densiflora* leaves according to the procedure, which is described in Fig. 1. A 4 % (w/v) suspension of the powder was boiled in deionized water for 10 min at 92 °C. The insoluble material was collected on a G4 fritted glass crucible and then suspended in fresh deionized water to become 1.67 % (w/v).

The holocellulose fraction was prepared using sodium chlorite [15]. The required volumes and weights of components were scaled to the sample mass. The holocellulose fraction was collected by centrifugation and the precipitate was extracted with 10 % sodium hydroxide for 16 h at room temperature. The concentration of holocellulose in the alkaline solution was determined from an approximately 45 % recovery of holocellulose from the starting powder in a preliminary experiment. Insoluble material in the extract was removed by centrifugation and the supernatant was neutralized with dilute hydrochloric acid. The neutralized solution was dialyzed against deionized water using dialysis tubing (molecular weight cut off 3500; Fisher Scientific). The dialyzed solution was mixed with 4 volumes of ethanol (80 % ethanol), and after centrifugation

the precipitate was freeze-dried and designated as the hemicellulose fraction (AHC) from *P. densiflora* leaves. AHC was suspended in the cold water (2 %, w/v) and stirred to separate the water-soluble fraction (AHC-X) from the water-insoluble fraction (AHC-M). Subsequently, AHC-X and AHC-M fractions were separated by centrifugation and freeze-dried.

Size exclusion chromatography (SEC) of hemicellulose

Molar mass distributions of the hemicellulose samples were analyzed by SEC using two KD-806M columns (300 × 7.5 mm, Shodex) connected in series with a guard column and equilibrated with dimethylsulfoxide (DMSO)/dimethylformamide (DMF) (80/20, v/v) containing 20 mM of lithium bromide (LiBr) and 20 mM of phosphoric acid [16]. The flow rate was 0.5 ml/min at 50 °C. The samples were filtered using PVDF membranes (0.45 μm) before analyses. The elution patterns were monitored using a differential refractometer, and the molar mass data were calculated using dextran standards by LC solution GPC software (Shimadzu).

Preparation of films

Polystyrene petri dishes, 90 mm in diameter, were used to prepare the hemicellulose films. The suspensions were adjusted to 127.1 mg/7.5 ml (2 mg/cm²) of hemicelluloses per unit of petri dish surface area. The suspensions were heated for 5 min in a boiling water bath to enhance dissolution and dispersion before use. For sample with soybean lecithin, a 1 % lecithin solution dissolved in 40 % ethanol was used to adjust the concentration before boiling. After allowing the suspensions to cool down to room temperature, they were gently mixed and cast into petri dishes. The films were dried at 24 °C and then analyzed.

Tensile testing

The tensile strength and elongation at break for the films were assessed at 22 °C and 50 % RH using a Shimadzu material testing machine (EZ-Graph) with a 500 N load cell. The specimens were aged under these conditions for at least 2 days prior to testing. The initial gauge length was 10 mm, and the crosshead speed was 1 mm/min. The width of the specimen was 10 mm, and the length was not less than 40 mm. The results are expressed as the means of five specimens.

Light transmittance

The light transmittance of each film was determined from 220 to 850 nm using a Shimadzu UV-2400 PC

Table 1 Relative sugar compositions of hemicellulose fractions from *Pinus densiflora* leaves

Components	Relative sugar amount (mol%)		
	AHC	AHC-X	AHC-M
Ara	5.7	6.4	8.8
Rha	1.4	1.2	1.6
Fuc	0.7	0.5	1.0
Xyl	17.5	25.6	9.4
Man	22.6	18.3	29.3
Gal	10.1	10.8	7.6
Glc	21.2	17.5	28.1
GlcUA	0.9	0.6	0
GalUA	7.6	2.5	10.8
MeGlcUA	12.2	16.5	3.3

spectrophotometer equipped with an integrating sphere attachment ISR-2200 (Shimadzu).

Results and discussion

Major constituent analyses of *P. densiflora* needle powder

The particle size distribution of *P. densiflora* powder was 32.75 μm as the value-based median value (D50). D50 was defined as the diameter at which half of the population lies below this value. The dry leaf powder was composed of 28.1 % ethanol extract fraction, 1.4 % ash, 26.3 % acid-insoluble lignin, and polysaccharides (44.2 %). The amount of holocellulose, prepared using sodium chlorite, was approximately 45 % from the starting powder. Thus, the amount of polysaccharides contained in the *P. densiflora* leaves should be 44–45 %.

Hemicellulose from *P. densiflora* leaves

According to the method for preparing hemicellulose fractions, as described in Fig. 1, 12.3 g (11.8 g DM) of AHC was obtained from 120 g (115.4 g DM) of the leaf powder, which was a 10.2 % yield from the original dry material.

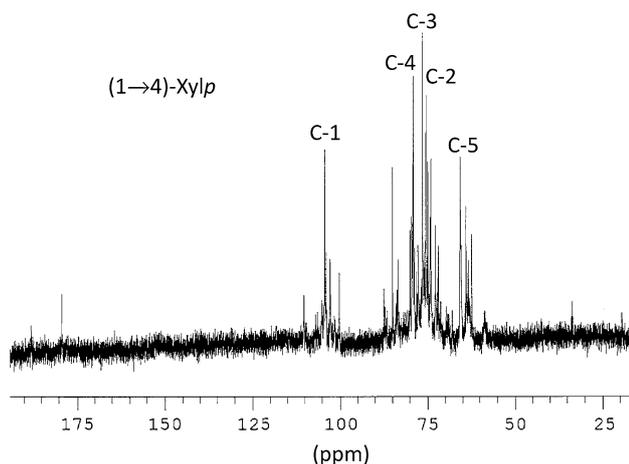
The ash and acid-insoluble lignin contents of AHC were 6.68 and 11.2 %, respectively. The ash content of AHC was a little higher than that of the ethanol-extracted powder (2.0 %), which could be a consequence of the residual salt generated by the neutralization process that was not removed by dialysis before the freeze-drying process.

The AHC fraction was separated into AHC-X and AHC-M according to their solubilities in cold water. The yield of AHC-X from AHC was 69 % and that of AHC-M was

Table 2 Peak assignments of ^{13}C -NMR spectra of water-soluble fraction (AHC-X) from hemicellulose from *Pinus densiflora* leaves

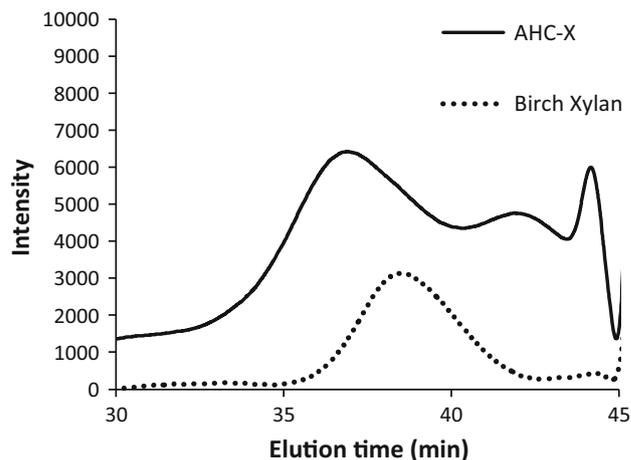
Glycosyl residues	Chemical shift ^a						
	C-1	C-2	C-3	C-4	C-5	C-6	OCH ₃
(1 → 4)-Xylp	102.47	73.52	74.54	77.22	63.81		
(1 → 4)-Xylp-2-O-GlcUA	102.10	76.92	73.05	76.92	63.68		
4-O-Me-GlcUA	98.39	72.17	77.65	83.19	73.13	177.40	60.52
Araf	108.45	81.60	78.08	85.57	62.22		

^a In ppm relative to the signal of internal dioxane in D₂O at 67.40 ppm

**Fig. 2** The ^{13}C -NMR spectrum of the water-soluble fraction (AHC-X) from hemicellulose from *Pinus densiflora* leaves, recorded relative to internal TSP

31 %. The relative sugar compositions of AHC, AHC-X, and AHC-M showed that the major sugar component of AHC-X is a xylan-related polysaccharide, whereas that of AHC-M is a mannan- and glucan-related polysaccharide (Table 1).

Table 2 shows the peak assignments of the ^{13}C -NMR spectra of AHC-X based on spectrum data from (arabino)-4-*O*-methylglucuronoxylan [17–19]. The data from arabinans and arabinoxylans were also referred [20, 21]. The presence of predominant (1-4)- β -linked xylan backbone was supported by peaks at $\delta_{\text{C}} = 63.81$ (65.81), 73.52 (75.50), 74.54 (76.52), 77.22 (79.21), and 102.47 (104.47) in ppm relative to dioxane (TSP) (Fig. 2). These assignments were supported by the results obtained from the methylation analysis, with the presence of 4-linked Xyl and 3-linked Ara. The AHC-X fraction included arabino-4-*O*-methylglucuronoxylan as a major component, and the mannan-related polysaccharide and arabinan were also suggested as mixed constituents. The results of the

**Fig. 3** Size exclusion chromatogram of the water-soluble hemicellulose fraction from *Pinus densiflora* leaves (AHC-X, solid line) and birch xylan (dotted line)

methylation analysis in this study showed higher amounts of 2, 3, 4-linked Xyl, which sometimes intimated the inadequate reaction. Thus, the fine structure of the components should be reinspected after purifying each element.

Arabino-4-*O*-methylglucuronoxylan, galactoglucomanan, and glucomannan are consistent elements of softwood hemicellulose [22]. The major component of hemicellulose from the trunk of *P. densiflora* is reportedly acetyl galactoglucomannan [23]. Glucomannan and arabinoglucuronoxylan are reportedly major components of hemicellulose in the water-soluble polysaccharide fraction from the root of *P. densiflora* [24]. This study presents some compositional differences between hemicelluloses from the trunk and the leaves of *P. densiflora*.

The molar mass distribution of AHC-X is shown in Fig. 3. AHC-X was easily dissolved in DMSO:DMF (80:20) containing 20 mM of LiBr and 10 mM of phosphoric acid, whereas AHC showed slight turbidity in the solvent. AHC-M showed apparent insoluble material in the solvent, thus the data of AHC and AHC-M were not shown in this study. Saake et al. [25] presented a solvent system of DMSO:water (90:10) with addition of 20 mM of LiBr, as a good mobile phase for xylan analysis. However, because the SEC columns in our study could not withstand the designated percentage of water, we selected a different solvent system. Two solvent systems did not show a significant difference in the apparent solubility of the AHC series, which means that AHC and AHC-M could not completely dissolve in DMSO:water (90:10) in the presence 20 mM of LiBr. The estimated weight-average molar mass value (M_w) of AHC-X was 50000. The M_w of birch xylan calculated by this system was estimated to be 32,000 and that of beech xylan was 21000 (data not shown).

Consequently, hemicellulose from *P. densiflora* leaves presented higher molar mass distribution than those of birch and beech xylans. The soluble parts from AHC and AHC-M showed even higher molar mass distributions than that of AHC-X (data not shown).

Film preparation from AHC series

Translucent films were formed by casting and drying the AHC suspensions on petri dishes. Although self-supporting films with AHC contents of 0.5 and 1.0 mg/cm² could be peeled from the dishes, the instrument for tensile testing required the film to be prepared with an AHC content of ≥ 2.0 mg/cm². The average thickness of film prepared with an AHC content of 2.0 mg/cm² was 12 μ m. The AHC-X fraction produced a more transparent film than the AHC fraction. However, the film from AHC-X was difficult to remove from the dishes without any additives such as plasticizer or separating compound. Some previous investigations suggested the addition of 10–40 % of plasticizer, (w/w of polysaccharide) of glycerol or sorbitol to obtain self-supporting films from xylan [7, 8]. Addition of these plasticizers, glycerol or sorbitol to the AHC-X solution resulted in a sticky self-supporting film that was easily peeled from the dish. To reduce the large amount of additives we used a method for oblate preparation, in which a small amount of edible oil and lecithin from soybean were included to improve the flexibility of the film and enhance detachability from a surface [26]. Lecithin from soybean was added to the AHC-X solution at various concentrations. When 0.5 % (w/w of AHC-X) lecithin was added, the film was difficult to peel from the dish; therefore, 1 % of lecithin was added. The film prepared from AHC-M was easily peeled from the dishes and required no additives. The films from AHC-M were most opaque and cracks were prevalent along the edges of the dishes. The whole hemicellulose fraction (AHC) was the best manageable fraction to obtain thin self-supporting films without additives. The polysaccharide component ratio of AHC might be in good order to form self-supporting film. The films from AHC-X and AHC-M prepared using 2 mg/cm² of hemicellulose met the conditions necessary for tensile testing. The average film thicknesses of AHC-X and AHC-M were 12 and 10 μ m, respectively.

Properties of films from the AHC series

The mechanical properties of the films were evaluated using tensile testing. Figure 4 shows typical stress–strain curves of each one of the five specimens. The films from *P. densiflora* leaves hemicellulose were strong with stress at break around 35 MPa and flexible with an elongation at break of around 2 %. The tensile strength and elongation at

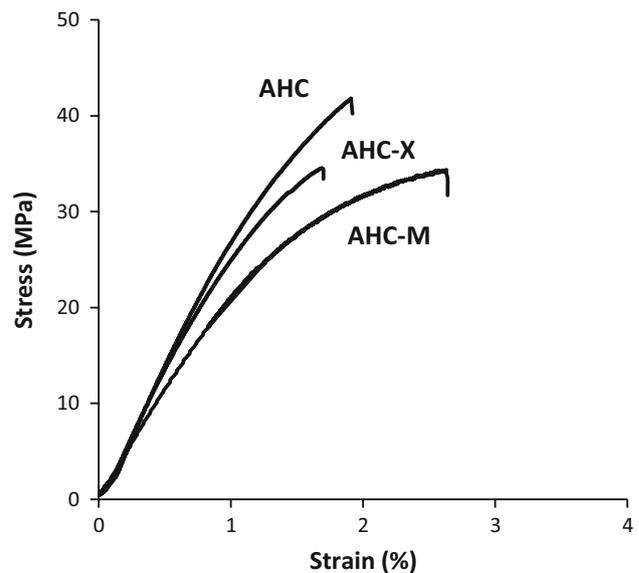


Fig. 4 Stress–strain curves of hemicellulose films from *Pinus densiflora* leaves. Typical stress–strain curve of each one of the five specimens is shown

Table 3 Tensile strength and elongation at break of the hemicellulose films from *P. densiflora* leaves

Hemicellulose fractions	Tensile strength (MPa)	Elongation at break (%)
AHC	42.51 \pm 5.50	2.19 \pm 0.64
AHC-X	35.70 \pm 13.03	2.47 \pm 0.76
AHC-M	36.61 \pm 5.35	2.14 \pm 0.42

Mean values \pm SD ($n = 5$) are shown

break of films from AHC, AHC-X (with 1 % lecithin), and AHC-M are summarized in Table 3. No significant differences in tensile properties were observed between the three fractions. The average tensile strength of AHC was 43 MPa, which was lower than that previously reported for xylan films without additives (52 MPa from rye endosperm arabinoxylan film; a 20–25 μ m thick film [27] and 50 MPa from barley husk arabinoxylan film; a 49 \pm 7- μ m thick film [28]). No significant difference in elongation at break was observed between the three fractions. The average elongation at break of films from the AHC series ranged between 2 and 2.5 %. A decrease in tensile strength and an increase in elongation at break are observed when the amount of plasticizer additives is increased [6, 7, 9]. Thus, previous reports suggest that by selecting appropriate additives, the properties of hemicellulose films from *P. densiflora* leaves can be improved.

The AHC film had a light transparency of ≥ 80 % at wavelength greater than 449 nm. Similarly, the AHC-X and AHC-M films had higher transparencies at wavelengths

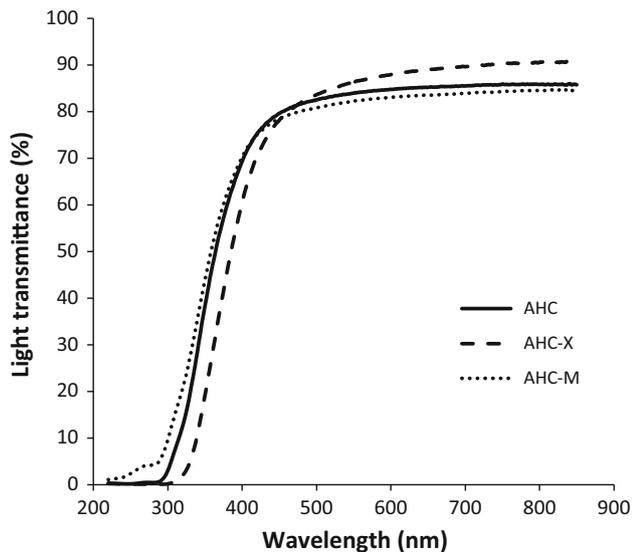


Fig. 5 Light transmittance of hemicellulose films from *Pinus densiflora* leaves. Solid line AHC, broken line AHC-X, dotted line AHC-M

>459 and >467 nm, respectively (Fig. 5). In the long-wavelength region, the light transmission was high in the order of AHC-X, AHC, and AHC-M. The film from AHC-X may have higher potential as a transparent material than films from AHC and AHC-M.

Conclusions

Translucent and transparent films were prepared using the hemicellulose fractions from the leaves of *P. densiflora* without substantial amounts of additives. The beneficial use of sustainable bioresource material to replace petroleum-based material has become increasingly important. The properties of the hemicellulose films from in this study are comparable to xylan films, and hence they may have potential as edible or biodegradable films. *P. densiflora* leaves have a history of being edible; thus, the leaves of this tree have high potential to produce a useful and edible hemicellulose film.

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References

- Arai M, Fujimoto K, Hashimoto T, Takishima T, Koya S (1987) Studies on the constituents of *Pinus densiflora* Sieb. et Zucc. leaves: on the ethanol-extractable amino acids (in Japanese). *Yakugaku Zasshi* 107:279–286
- Ogasawara R (1974) Changes of chemical constituents in leaves of *Pinus densiflora* trees according to tree age and leaf age (in Japanese). *Fac Agr Tottori Univ* 56:271–275
- Yokouchi Y, Ambe Y, Fuwa K (1981) The relationship of the monoterpene composition in the atmosphere, the foliar emission gas and the leaf oil of *Pinus densiflora*. *Chemosphere* 10:209–213
- Kim YS, Shin DH (2005) Volatile components and antibacterial effects of pine needle (*Pinus densiflora* S. and Z.) extracts. *Food Microbiol* 22:37–45
- Jo JR, Park JS, Park YK, Chae YZ, Lee GH, Park GY, Jang BC (2012) *Pinus densiflora* leaf essential oil induces apoptosis via ROS generation and activation of caspases in YD-8 human oral cancer cells. *Int J Oncol* 40:1238–1245
- Mikkonen KS, Tenkanen M (2012) Sustainable food-packaging materials based on future biorefinery products: xylans and mannans. *Trends Food Sci Technol* 28:90–102
- Mikkonen KS, Heikkinen S, Soovre A, Peura M, Serimaa R, Talja RA, Helén H, Hyvönen L, Tenkanen M (2009) Films from oat spelt arabinoxylan plasticized with glycerol and sorbitol. *J Appl Polym Sci* 114:457–466
- Zhang P, Whistler R (2004) Mechanical properties and water vapor permeability of thin film from corn hull arabinoxylan. *J Appl Polym Sci* 93:2896–2902
- Gröndahl M, Eriksson L, Gatenholm P (2004) Material properties of plasticized hardwood xylans for potential application as oxygen barrier films. *Biomacromolecules* 5:1528–1535
- Sadoh T (1962) Study on the sorption of water vapor by softwood hemicelluloses. *Sci Rep Kyoto Pref Univ Agr* 14:99–105
- Effland MJ (1977) Modified procedure to determine acid-insoluble lignin in wood and pulp. *Tappi* 60:143–144
- York WS, Darvill AG, McNeil M, Stevenson TT, Albersheim P (1985) Isolation and characterization of plant cell walls and cell wall components. *Methods Enzymol* 118:3–40
- Sanford PA, Conrad HE (1996) The structure of the *Aerobacter aerogene* A3 (S1) polysaccharide I: a re-examination using improved procedure for methylation analysis. *Biochemistry* 5:1508–1516
- Hakomori S (1964) A rapid permethylation of glycolipid and polysaccharide catalyzed by methylsulfinyl carbanion in dimethyl sulfoxide. *J Biochem* 55:205–208
- Browning BL (1967) Holocellulose by the chlorine dioxide and chlorite methods. In: *Methods of wood chemistry II*. Interscience publishers, New York, pp 393–396
- Technical report for GPC of xylan using a LF804 column (in Japanese). <http://www.shodex.com/ja/dc/06/09/19.html>. Accessed 23 July 2014
- Shi ZJ, Xiao LP, Deng J, Yang HY, Song XL, Sun RC (2013) Isolation and structural exploration of hemicelluloses from the largest bamboo species: *Dendrocalamus sinicus*. *BioResources* 8:5036–5050
- Dinand E, Vignon MR (2001) Isolation and NMR characterization of a (4-O-methyl-D-glucurono)-D-xylan from sugar beet pulp. *Carbohydr Res* 330:285–288
- Habibi Y, Mahrouz M, Vignon MR (2002) Isolation and structure of D-xylans from pericarp seeds of *Opuntia ficus-indica* prickly pear fruits. *Carbohydr Res* 337:1593–1598
- Roubroeks JP, Andersson R, Åman P (2000) Structural features of (1 → 3), (1 → 4)-β-D-glucan and arabinoxylan fractions isolated from rye bran. *Carbohydr Res* 42:3–11
- Dourado F, Cardoso SM, Silba AMS, Gama FM, Coimbra MA (2006) NMR structural elucidation of the arabinan from *Prunus dulcis* immunobiological active pectic polysaccharides. *Carbohydr Res* 66:27–33
- Shimizu K (1991) Chemistry of hemicelluloses. In: Hon DNS, Shiraiishi N (eds) *Wood and cellulosic chemistry*. Dekker, New York, pp 177–214

23. Koshijima T, Tanaka R (1971) Fractionation of the lignin-containing hemicellulose extracted with dimethylsulfoxide from Japanese red pine. *Cellulose Chem Technol* 6:609–620
24. Watanabe T, Inaba K, Nakai A, Mitsunaga T, Ohnishi J, Koshijima T (1991) Water-soluble polysaccharides from the root of *Pinus densiflora*. *Phytochemistry* 30:1425–1429
25. Saake B, Kruse T, Puls J (2001) Investigation on molar mass, solubility and enzymatic fragmentation of xylans by multi-detected SEC chromatography. *Biores Tech* 80:195–204
26. Tsunoda K (2011) Oblate production using the potato starch from Hokkaido (in Japanese). http://www.alic.go.jp/joho-d/joho08_000096.html. Accessed 23 July 2014
27. Höije A, Sternemalm E, Heikkinen S, Tenkanen M, Gatenholm P (2008) Material properties of films from enzymatically tailored arabinoxylans. *Biomacromolecules* 9:2042–2047
28. Höije A, Gröndahl M, Tømmeraas K, Gatenholm P (2005) Isolation and characterization of physicochemical and material properties of arabinoxylans from barley husks. *Carbohydr Polym* 61:266–275