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STEPWISE EXTRACTION OF HEMICELLULOSES WITH WATER AND ALKALI FROM LARCH WOOD AND THEIR SUGAR COMPOSITIONS

XIAOYAN ZHANG, LANXING DU, SIJIN LI
HEBEI AGRICULTURAL UNIVERSITY
CHINA

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ABSTRACT

The aim of the present study was to isolate hemicelluloses by stepwise extraction with water and alkali from larch (*Larix principis-rupprechtii* Mayr) sapwood and heartwood. One water-soluble arabinogalactan (AG) and three alkali-soluble hemicelluloses-arabinoglucuronoxylan (AGX), galactoglucomannan (GGM) and glucomannan (GM) were obtained. The yield of AG extracted with hot-water from larch heartwood was 7.57%, it was 17.96% in total of three alkali-extracted hemicelluloses. There was no significant difference in the yield of hemicelluloses from sapwood and heartwood. Monosaccharide compositions of the hemicelluloses were determined by high performance liquid chromatography after acid hydrolysis. The results showed that galactose and mannose were the main glycosyl units of hemicellulose, followed by xylose. Galactose mainly derived from AG, whereas mannose and xylose originated from alkali-extracted hemicelluloses.

KEYWORDS: Hemicellulose, extraction, water, alkali, monosaccharide.

INTRODUCTION

Hemicellulose is one of the three major components of lignocellulosic biomass, its reserves are second only to cellulose in nature, accounting for about 20–30 wt% (Timell and Syracuse 1967, Lu et al. 2021). Hemicellulose is a complex branched carbohydrate bio-polymer composed of hexoses (β-D-mannose, β-D-glucose, β-D-galactose), pentoses (β-D-xylose and α-D-arabinose), glucuronic acid, and a small amount of L-rhamnose and L-fucose units. Hemicellulose is a renewable, biodegradable, and environmentally friendly biomass resources, it has a broad application prospect in chemical, food, papermaking, bio-medicine, and coating

industries. It has been reported that oligosaccharide (Jayapal et al. 2013, Francillon et al. 2020), xylose (Hricovíniová 2013), xylitol (Yi and Zhang 2012, Dietrich et al. 2017), furfural (Hricovíniová 2013), ethanol (Agbogbo and Wenger 2007), lactic acid (Walton et al. 2010, Campos et al. 2021) and other products are produced from hemicellulose.

Hemicellulose is closely bound to cellulose and lignin together in plant cell-wall, it connected to cellulose through hydrogen bonding and van der Waals forces, and formed lignin-carbohydrate complexes (LCC) with lignin by chemical bonding. The premise of efficient utilization of hemicellulose is to extract and separate hemicellulose from lignocellulosic resources. The extraction and separation of hemicellulose from lignocellulosic biomass include hydrothermal extraction (Song et al. 2011, Song et al. 2012, Gullon et al. 2012, Gonzalez-Munoz et al. 2013, Azhar et al. 2015, Chadni et al. 2019), alkaline extraction (Wei et al. 2013), acidic extraction (Zhou et al. 2017, Song et al. 2020, Spronsen et al. 2011), steam explosion (Jedvert et al. 2012, Xing et al. 2014), solvent extraction (Spronsen et al. 2011, Froschauer et al. 2013, Leskinen et al. 2013), ultrasonic-assisted and microwave-assisted processes (Markin et al. 2015, Zhou et al. 2017, Liu et al. 2018, Chadni et al. 2019), and freeze-thaw repetition assisted method (Zhu et al. 2020). The hydrogen bonds between hemicellulose and cellulose and the chemical bonding between hemicellulose and lignin were destroyed in alkaline media. In addition, hemicellulose has a higher dissolution rate than that of cellulose, and a more powerful antioxidant property than that of lignin in alkaline solution (Egüés et al. 2012). Owing to high degree of polymerization, less degradation of sugars, hemicellulose extracted with alkali is beneficial for further utilization (Chadni et al. 2019).

North China larch (*Larix principis-rupprechtii* Mayr) is the main forest tree species in North China, it distributes in mountainous areas with an altitude of 1.4–1.8 km. It has huge reserves because of fast growth, strong resistance to adverse weather, and the effect of soil conservation. It is reported that members of the genus *Larix* woods contained 10–25% of a water-soluble hemicellulose–arabinogalactan, which in other softwood was in only trace amounts (Timell and Syracuse 1967). In the present study, hemicelluloses were extracted with water and alkali from *Larix principis-rupprechtii* wood, and then they were hydrolyzed by sulfuric acid for determination of the monosaccharide composition and proportion.

MATERIALS AND METHODS

Materials

In this study, twenty year-old larch (*Larix principis-rupprechtii* Mayr) logs were provided by Saihanba Machinery Forest Farm (Hebei province, China). The sapwoods and heartwoods were crushed into powder of 40–60 mesh respectively and placed for 12 h in a 105ºC oven for drying to a constant weight.

Determination of chemical compositions

The cellulose content was determined using nitric acid and ethanol mixture (Chen et al. 1990). The materials were treated by nitric acid ethanol mixture (ratio 1:4) 4 times in boiling

water bath, 1 h at a time. The remaining cellulose was rinsed thoroughly and dried at 105ºC. The cellulose content was calculated by the dry weight of cellulose (g)/dry weight of material (g). The benzene-ethanol extractive was the percentage content of substances extracted from benzene-ethanol mixture (ratio 2:1) for 6 h (GB/T 35816, 2018).

Sodium chlorite was used to determine the content of holocellulose (GB/T 35818, 2018). The materials extracted from benzene-ethanol mixture were treated by sodium chlorite (0.3 g/g material) and glacial acetic acid (0.25 mL/g material) 4 times in 75ºC of constant-temperature water bath, 1 h at a time. The holocellulose remained was washed thoroughly and dried at 105ºC. The holocellulose content was calculated by the dry weight of holocellulose (g)/dry weight of material (g).

The lignin content was determined by sulfuric acid method (GB/T 35818, 2018). The materials extracted from benzene-ethanol mixture were hydrolyzed by 72% sulfuric acid for 2 h at room temperature. The concentration of sulfuric acid was then reduced to 3% to continue the hydrolysis for 4 h at 100ºC. The lignin content was calculated using the ratio dry weight of lignin (g)/dry weight of material (g).

Extraction of water-soluble hemicellulose

The materials used were extracted by benzene-ethanol mixture in advance. Conditions of 1:150 solid-liquid ratio, 23 ± 2ºC temperature, 2 days extraction time were used for cool-water extraction, and 1:100, 100ºC, 3 h were used for hot-water extraction. The mixture was shaken frequently during extracting. The extract solution was depressurized and concentrated to a certain volume, and impurities were removed by centrifugation. 3 times the volume of ethanol was added to the extract solution, and the pH was adjusted to 3.9 until white flocculent sediment appeared. The precipitate obtained by centrifugation was washed completely with ethanol and ether, and was dried at room temperature and then dried to constant weight in an oven at 105ºC. The hemicellulose extracted with water was arabinogalactan (AG).

Extraction of alkali-soluble hemicelluloses

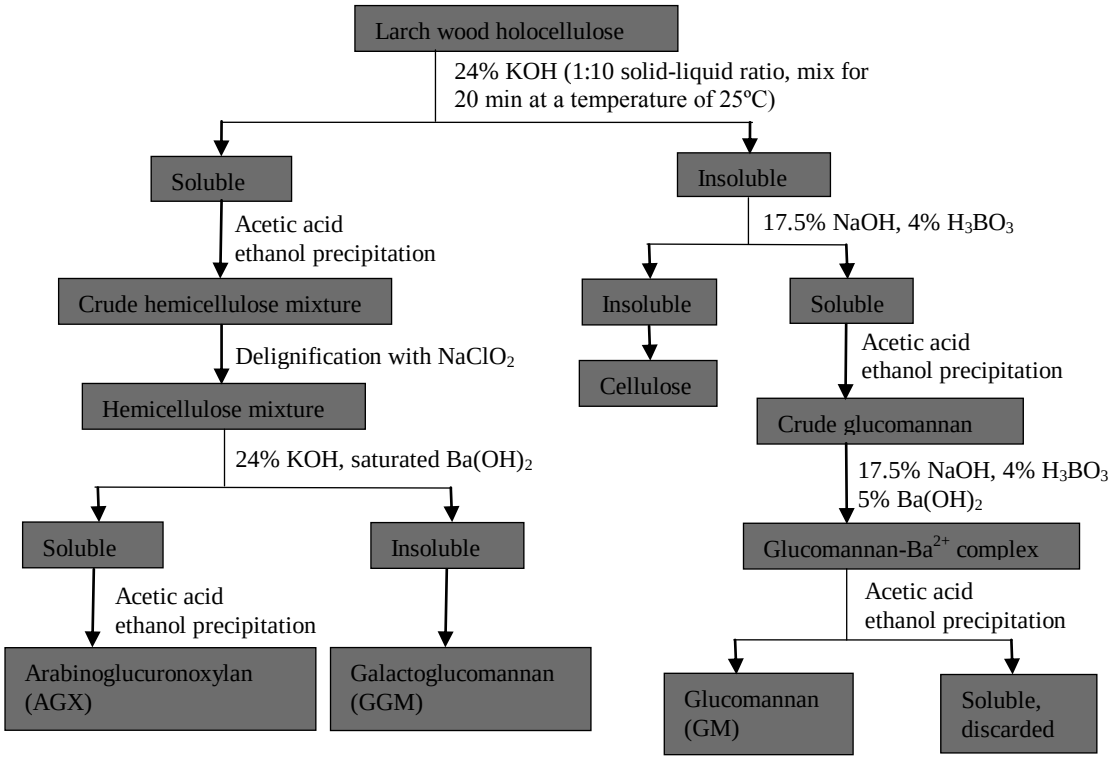

Fig. 1: The operation process of stepwise extracting alkali-soluble hemicelluloses from larch wood holocellulose.

In this study, holocelluloses used were extracted by hot water in advance. According to the method of Timell and Syracuse (1967), that is, multi-step extraction of different alkalis, acidification and ethanol precipitation to separate hemicelluloses from holocellulose. The hemicelluloses extracted with different alkalis were arabinoglucuronoxylan (AGX), galactoglucomannan (GGM) and glucomannan (GM). The extraction process was shown in Fig. 1.

Hemicellulose hydrolysis

Acid hydrolysis method was used (Li and Lu 1985). 0.1 g of hemicellulose was soaked and ground for 45 min using 0.5 mL 77% sulfuric acid at a temperature of 25ºC. The concentration of sulfuric acid was then reduced to 3%, and was boiled and refluxed for 4 h to allow hydrolysis to continue. The pH was adjusted to 5.0 with saturated barium hydroxide solution when the hydrolysate cooled down. After that, the barium sulfate precipitation was removed by centrifugation. The hydrolysate was evaporated and concentrated to make the sugar concentration reach 10 g/L, and was stored in the refrigerator in dark.

Determination of sugar concentration

High-performance liquid chromatography (HPLC, model: waters 2695) was conducted to determine the concentrations of glucose, galactose, mannose, xylose, and arabinose in hydrolysate at 30ºC, with acetonitrile and water (with a ratio of 79:21) as the mobile phase (flow

rate 1.0 mL·min^{-1}). The HPLC has an Evaporative Light Scattering (ELS) detector with a column of Xbridge Amide Nucleosil 100-5 NH_2 4.6×250 mm, 3.5 μm, made in waters corporation, America.

Yield calculation

The water extract was calculated by the loss weight of the material after extraction (g)/dry weight of material before extraction (g). The yield of AG was determined by the dry weight of AG (g)/dry weight of material (g). The recovery of AG was calculated using the ratio AG yield (%)/water extract (%). The yield of alkali-extracted hemicelluloses (AGX, GGM, or GM) from holocelluloses was calculated using the ratio dry weight of AGX, GGM, or GM (g)/dry weight of holocelluloses (g). The yield of AGX, GGM, or GM from wood was determined by the AGX, GGM, or GM yield from holocellulose (%) × holocellulose percentage content in wood (%). The yield of monosaccharide was determined by the yield of hemicellulose from wood (%) × the monosaccharide percentage in hydrolysate of hemicellulose (%).

RESULTS AND DISCUSSION

Chemical compositions of larch wood

The relative percentage contents of chemical compositions in larch sapwood and heartwood are presented in Tab. 1. As shown in the table, the relative percentage contents of lignin and extractive in sapwood and heartwood had minor difference, and they were close to that obtained by Wang et al. (2004) for study on chemical compositions of *Larix kaempferi*. The cellulose content in heartwood was slightly lower than that in sapwood. The possible reason was that a part of heartwood (near the pith) was juvenile wood, which had lower cellulose content than transition wood (Sykes et al. 2006). A small amount of lignin remained in holocellulose, because some hemicellulose and lignin formed lignin-carbohydrate complexes (LCC), they connected by chemical bond. The content of hemicellulose was the value by subtraction cellulose and residual lignin content from holocellulose content. The hemicellulose content was lower than actual content in wood, because a part of water-soluble hemicellulose (such as arabinogalactan) was lost during the preparation of holocellulose. 23.34% hemicellulose content (closer to this study) in research of Wang et al. (2016) on chemical compositions of larch wood also confirmed this viewpoint.

Tab. 1: Chemical compositions in larch sapwood and heartwood.

Sample	**Cellulose** (%)	**Holocellulose (Residual lignin)** (%)	**Hemicellulose** (%)	**Lignin** (%)	**Benzene-ethanol extractive** (%)
Sapwood	45.60	71.08 (2.85)	22.63	28.06	3.09
Heartwood	44.26	73.14 (4.16)	24.72	27.81	3.21

*Values are means of duplicate. Hemicellulose (%) = Holocellulose (%) - Residual lignin (%) - cellulose (%).

Yield of water-extracted hemicellulose and its monosaccharide composition

The water extracts, yield of water-soluble arabinogalactan (AG), recovery rate of AG are

listed in Tab. 2. The relative contents of water extracts ranged from 9% to 11%, and the hot-water extracts were slightly higher than cool-water extracts in both sapwood and heartwood. The recovery of AG precipitated with ethanol from cool-water extracts account for 71.76% in sapwood and 73.95% in heartwood, higher than that (67.36% in sapwood and 70.03% in heartwood) from hot-water extracts. The higher hot-water extracts and lower AG recovery explained that AG degraded more in hot water than that in cold water. The yield of AG extracted from cool or hot water was about 7 g per 100 g materials of sapwood or heartwood. The findings in this research were almost identical with the reports to wood of *Larix gmelinii* by Li and Ji (1994).

Tab. 2: Water extract, yield and recovery rate of AG in larch sapwood and heartwood.

Extractions	**Water extract** (%)	**Yield of AG** (g / 100 g woods)	**Recovery of AG** (%)
Sapwood, cool-water	9.10	6.53	71.76
Sapwood, hot-water	10.14	6.83	67.36
Heartwood, cool-water	9.52	7.04	73.95
Heartwood, hot-water	10.81	7.57	70.03

*Water extract (%) was expressed as a loss rate of dry weight of material. Yield of AG (%) = dry weight of AG (g)/dry weight of material (g). Recovery of AG (%) = AG yield (%)/Water extractive (G).

Two monosaccharides of arabinose and galactose were found in AG hydrolysate by HPLC. The percentage contents of arabinose were only about 5%, far lower than about 95% of galactose, as illustrated in Tab. 3. The molar ratio of galactose to arabinose was 15:1 for sapwood and 17:1 for heartwood, both were much higher than 6:1 or 8:1 in other's reports (Timell and Syracuse 1967, Wu et al. 1987, Scheller and Ulvskov 2010). The yield of arabinose from AG hydrolysis ranged from 0.32 g to 0.35 g per 100 g woods, however, the yield of galactose was as high as 6.20 – 7.23 g per 100 g woods (Tab. 3).

Tab. 3: Monosaccharide percentage in AG hydrolysate *and yield of monosaccharide from larch sapwood and heartwood.*

Extractions	**Monosaccharide percentage in AG hydrolysate** (%)		**Yield of monosaccharide** (%)	
	Arabinose	**Galactose**	**Arabinose**	**Galactose**
Sapwood, cool-water	5.02	94.98	0.33	6.20
Sapwood, hot-water	5.10	94.90	0.35	6.48
Heartwood, cool-water	4.53	95.47	0.32	6.72
Heartwood, hot-water	4.51	95.49	0.34	7.23

*Values are means of duplicate. Yield of monosaccharide (%) = AG yield (%) × monosaccharide percentage in AG hydrolysate (%).

Larch AG is constituted by a β-1,3-galactan main chain with β-1,6-galactan side chains, to which α-L-arabinofuranosyl and β-L-arabinopyranosyl residues are attached, there are no arabinose branches attached directly to the main chain. The branching and arabinose content increase with the size of side chains (Ponder and Richards 1997). The ratio of galactose and arabinose was changed from 7:3 to 16:1 when larch AG, extracted from *Larix gmelinii* sawdust, was depolymerized by H_2O_2 oxidation (Cheng et al. 2021). The higher ratio of galactose to arabinose in this study showed that some changes may have taken place in the extraction of AG

or during the ethanol precipitation process. Because the extraction solution was adjusted to pH=3.9 when ethanol precipitation occurred, such low pH value could lead to the hydrolysis of arabinose groups on the AG branch chains and lost.

Yield of alkali-extracted hemicelluloses and monosaccharide compositions

Three alkali-soluble hemicelluloses (AGX, GGM, and GM) were extracted from larch sapwood and heartwood with different alkaline solutions. The yields of AGX, GGM, and GM from holocellulose and from wood were listed in Tab. 4. There no significant difference in total yield of alkali-extracted hemicellulose, 24.85% and 24.56% were the yield from holocellulose of larch sapwood and heartwood, 17.67% and 17.96% were the yield in sapwood and heartwood, respectively. There was a higher AGX content and a lower GGM content in heartwood than in sapwood, whereas the content of GM was roughly equivalent in heartwood and sapwood.

Tab. 4: The yield of alkali-extracted hemicelluloses (AGX, GGM, and GM) in larch sapwood and heartwood.

Hemicellulose	**Yield from holocellulose** (g / 100 g holocelluloses)	**Yield from wood** (g / 100 g woods)
AGX in sapwood	8.73	6.21
GGM in sapwood	6.71	4.77
GM in sapwood	9.41	6.69
Total in sapwood	24.85	17.67
AGX in heartwood	10.24	7.49
GGM in heartwood	5.10	3.73
GM in heartwood	9.22	6.74
Total in heartwood	24.56	17.96

*Values are means of duplicate. Yield from holocellulose (%) = dry weight of AGX, GGM, or GM (g)/dry weight of holocellulose (g). Yield from wood (%) = Yield from holocellulose (%) × holocellulose content in wood (%).

The relative percentage contents of sugars in hydrolysate of alkali-extracted hemicelluloses were illustrated in Fig. 2. Results showed that five monosaccharides were detected in each alkali-extracted hemicellulose hydrolysate. The xylose percentage contents in AGX hydrolysate were more than 70% whether in sapwood or heartwood, the ratio of arabinose to xylose was about 1:5. The glucuronic acid was not detected in AGX hydrolysate.

The backbone of GGM and GM is a linear (GM) or slightly branched (GGM) chain comprising 1,4-linked β-D-mannopyranose and β-D-glucopyranose units, whereas the 1,6-linked α-galactopyranosyl units are attached only to the mannose units (Timell and Syracuse 1967). Depending on the wood species and isolation methods, the molar ratio of glucose:mannose ranges from 1.9:10 to 2.6:10 (Scheller and Ulvskov 2010). In this study, the molar ratios of GGM-derived galactose, glucose, and mannose were 0.3:1:2.8 in sapwood and 0.2:1:3.4 in heartwood. The relative content of galactopyranosyl units was low compared to the ratio of 0.6:1:3.6, derived from GGM of spruce wood (Song et al. 2013). The ratio of glucose to mannose originated from GM were 1:3.8 in sapwood and 1:3.4 in heartwood, similar to the ratio of glucose to mannose derived from GGM.

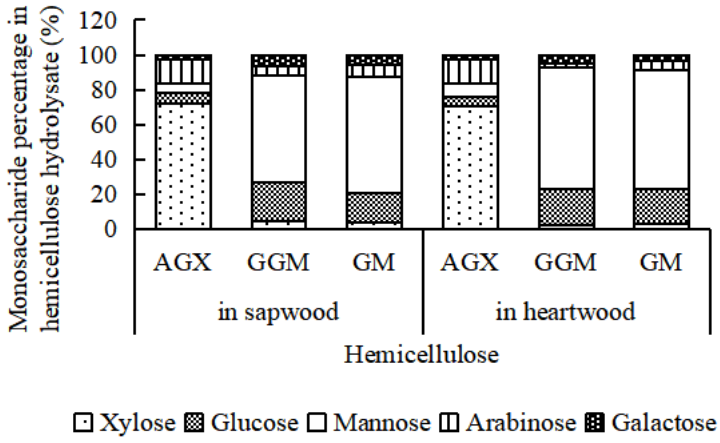

Fig. 2: Monosaccharide percentage in hydrolysate of alkali-extracted hemicelluloses.

The yields of five monosaccharides derived from alkali and water extracted hemicelluloses were listed in Tab. 5. Xylose, glucose, mannose, arabinose, and galactose yields were 4.90%, 2.62%, 7.72%, 1.94%, and 7.32%, respectively in sapwood, 5.54%, 2.52%, 7.79%, 1.83%, and 7.85%, individually in heartwood. The yield of galactose was more than twice that of *Larix kaempferi* wood, however, the mannose yield was only about 65% of what reported by Wang et al. (2004) (Tab. 6). The yields of xylose and arabinose were similar to or slightly higher than Wang's research (Wang et al. 2004).

Tab. 5: Yields of monosaccharides from hemicelluloses of larch sapwood and heartwood.

Hemicelluloses		**Xylose** (%)	**Glucose** (%)	**Mannose** (%)	**Arabinose** (%)	**Galactose** (%)
Sapwood	AGX	4.47	0.40	0.32	0.87	0.14
	GGM	0.20	1.06	2.96	0.23	0.31
	GM	0.24	1.16	4.43	0.48	0.38
	AG	—	—	—	0.35	6.48
	Total	4.90	2.62	7.72	1.94	7.32
Heartwood	AGX	5.30	0.37	0.58	1.02	0.22
	GGM	0.07	0.78	2.61	0.10	0.17
	GM	0.17	1.36	4.60	0.37	0.23
	AG	—	—	—	0.34	7.23
	Total	5.54	2.52	7.79	1.83	7.85

*Values are means of duplicate. Yield of monosaccharides (%) = hemicellulose yield (%) × monosaccharide percentage in hemicellulose hydrolysate (%).

Tab. 6: Yields of monosaccharides from hemicelluloses of different larch wood (%).

Sugars	***Larix principis-rupprechtii* wood in this study**		**Stem wood of *Larix kaempferi*[a]**		
	Sapwood	Heartwood	12-year	15-year	23-year
Xylose	4.90	5.54	6.18	6.46	4.51
Glucose	2.62	2.52	—	—	—
Mannose	7.72	7.79	12.19	11.35	12.94
Arabinose	1.94	1.83	1.46	1.55	1.22
Galactose	7.73	7.85	2.49	2.76	3.59

[a]Yields of monosaccharides from hemicelluloses of *Larix kaempferi* wood were taken from Wang et al. (2004).

CONCLUSIONS

Hemicelluloses can be extracted from larch (*Larix principis-rupprechtii*) wood in high yield with water and alkali. The yield of hemicelluloses extracted with water and alkali from larch sapwood and heartwood are 24.50% and 25.53% in total, respectively. In terms of the composition of hemicelluloses, galactose and mannose have the highest content, followed by xylose. Galactose mainly originated from water-soluble arabinogalactan, whereas mannose and xylose came from alkali-extracted hemicelluloses. It is worth mentioning that the obtained arabinogalactan has high purity, but its recovery rate was around 70%. The next focus of research is to improve the recovery rate of arabinogalactan to raise its yield.

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HEBEI AGRICULTURAL UNIVERSITY
COLLEGE OF FORESTRY
BAODING, P.R. CHINA
*Corresponding author: zhxyzi@163.com