Original article

Nacera Benouadah*, Andrey Pranovich, Djamel Aliouche, Jalel Labidi and Stefan Willför

Optimization of the extraction of galactoglucomannans from *Pinus halepensis*

https://doi.org/10.1515/hf-2020-0095
Received April 10, 2020; accepted September 23, 2020;
published online October 28, 2020

Abstract: The effectiveness of pressurized hot-water extraction conditions for obtaining galactoglucomannans (GGMs) from *Pinus halepensis* suitable for applications like coatings and films packaging was investigated. For this purpose, high molar masses with high yields are required, presenting a serious challenge for hot-water extraction processes. The extraction of GGMs was carried out in an accelerated solvent extractor (ASE) and the isolation was performed by precipitation in ethanol. Three temperatures in the range 160–180 °C and five extraction times 5–90 min were tested in order to optimize extraction parameters of GGMs, avoiding thermal and chemical degradation in hot-water. Total dissolved solids (TDS) were determined gravimetrically after freeze-drying and weight average molar masses (M_w) were determined by high-performance size exclusion chromatography (HPSEC). Total non-cellulosic carbohydrates were determined by gas chromatography (GC) after acid methanolysis. Free monomers were additionally analyzed by GC. Lignin in water extracts was measured by an ultraviolet (UV) method. Acetic acid was determined after alkaline hydrolysis of acetyl groups and analyzed by HPSEC. The main parameters influencing the extraction processes of the GGMs, namely, extraction time and temperature were studied. Optimal extraction parameters of GGMs were identified at 170 °C and 20 min extraction time, with average M_w of extracted fraction of 7 kDa leading to a GGM yield of approximately 56 mg $g^{-1}_{o.d.m}$, corresponding to 6% on dry wood basis.

Keywords: extraction; galactoglucomannans; optimization; *Pinus halepensis*.

1 Introduction

The needs of society to use sustainable sources of raw materials in the coming decades are necessary as the global economy interests become increasingly aware of the limited fossil fuels and the needs to move towards new alternative sources. In response to this challenge, lignocellulosic biomass-derived materials have been pointed out to be one of the most promising sources for many industrial processes (Zhou et al. 2006).

Lignocellulosic biomass consists mainly of structural components such as cellulose, hemicelluloses, lignin, pectins, and non-structural components such as lipophilic and polar extractives, tannins, waxes, sugar monomers and dimers, and inorganic salts (Laine 2005; Sjöström and Alén 1998). Hemicelluloses are the third most abundant wood polymer after cellulose and lignin and usually constitute between 20 and 30% of the total dry mass of wood (Sjöström 1993). Hemicelluloses are considered to be the main renewable source of sugars suited for various sugar-based biopolymers, including biocompatible hydrogels and biodegradable films (Ebringerova et al.1994; Gabrielii et al. 2000).

Galactoglucomannans (GGMs), also called glucomannans (GMs) for short, are the major hemicellulosic fraction present in softwood, mainly composed by galactose, glucose, and mannose units, which represent approximately 14–20% of the total dry mass of wood (Willför et al. 2005). There is a growing interest in utilizing GGMs for many applications, such as films and coatings, emulsifier and thickener in food additive, controlled drug delivery, papermaking, textile and cosmetics and for fuel production (Ebringerova et al. 2005; Willfor et al. 2008). GGMs can be used as long-chained and high-molar-mass

***Corresponding author: Nacera Benouadah**, Research Unit, Materials, Processes and Environment (UR-MPE), M'Hamed Bougara University, 35000 Boumerdes, Algeria,
E-mail: benouadahnacera@univ-boumerdes.dz
Andrey Pranovich and Stefan Willför, Johan Gadolin Process Chemistry Centre, Åbo Akademi University, Porthansgatan 3, FI-20500 Turku, Finland
Djamel Aliouche, Laboratory of Fibrous Polymers Treatment and Forming (LTMFP), M'Hamed Bougara University, 35000 Boumerdes, Algeria
Jalel Labidi, Environmental and Chemical Engineering Department, University of the Basque Country (UPV/EHU), Plaza Europa, 1, 20018 Donostia-San Sebastián, Spain

polymers for a variety of applications such as pharmaceuticals, cosmetics, and textiles, as well as alimentary and health products (Mikkonen et al. 2009; Rana et al. 2011). Besides these applications, low-molar-mass GGMs can be hydrolyzed to sugar monomers making them good candidates for processes of biofuel production and also serve as chemical platform for other applications (Gonzalez et al. 2011; Shevchenko et al. 2000). In the field of films and coatings, hemicellulose-based materials, such as various GGMs, xylans, and xyloglucans are under investigation involving chemical and physical cross-linking to improve their biocompatibility properties associated to their mechanical performances (Mikkonen et al. 2011; Trovatti et al. 2012).

Governing beneficial properties of GGM requires relatively high-molar-mass and the isolation and extraction techniques aiming at enhancing the yield of GGM extracts still need to be further innovated as a part of the bio-based materials processes. Average molar masses up to 20–60 kDa have been reported for GGM films using glyoxal-crosslinking as reinforcement (Mikkonen et al. 2012). New composite films of nanofibrillated cellulose for films packaging obtained from GGM coated with succinic esters of GGM have been suggested as suitable candidates for food packaging to replace typical oil-based non-biodegradable plastics currently used (Kisonen et al. 2015).

Hartman et al. (2006) used modified O-acetyl-galactoglucomannan (AcGGM) and benzylgalactoglucomannan (BnGGM) to produce oxygen barrier films exhibiting low moisture sensitivity. Other typical examples are mixtures of GGM and konjac glucomannan (KGM), mixtures of GGM/xanthan, GGM/guar gum, GGM/locust bean gum (LBG) and GGM/carrageenan (Xu et al. 2008), all of which have high-molar-masses, possess good effects on rheologic properties for modifying viscoelasticity, and synergistic interactions.

Chemical composition and structural properties of GGM depend on the isolation conditions and extraction techniques. The main challenge today remains in the ability to compromise the average molar mass and the total yield of GGM extracts according to specific requirements of such application avoiding their degradations into smaller oligomers or monomers in a technically suitable way. Several extraction methods for isolating hemicelluloses from wood have been investigated, and pressurized hot-water extraction has proven to be a very efficient method for extracting the hemicelluloses in an environmentally friendly way using water as solvent (Hosseinaei et al. 2011; Xu et al. 2009). Pranovich et al. (2015), studied the extraction of GGMs from spruce wood subjected to a series of sequential two-stage extractions using an ASE with the objective to preserve the polymeric structure and the acetylation degree of extracted GGMs and to achieve high yield. The study showed that the yield of GGMs with the weight average molar mass of 8–10 kDa during 160 °C extraction had a maximum at 20 min extraction time at 170 °C decreased to 6–2 kDa during the second hot-water extraction.

GGM has also been extracted from sapwood chips and ground wood of spruce at high yields by hot-water at 170–180 °C (Song et al. 2008). Much as 80–90% of the GGM in the wood was extracted from ground wood at 170–180 °C for an extraction time of 1 h. It has been shown that the optimized pH profile is a key factor for achieving a high yield of high-molar-mass GGM aiming to minimize hydrolysis of acetyl groups and hydrolytic cleavage of GGM chains.

The goal of this work was to study how the average molar mass and the yield of GGMs extracted from *Pinus halepensis* can be influenced by altering the experimental conditions during hot- water extraction. The main parameters influencing the extraction processes of the GGMs, namely, extraction time and temperature were studied in both water extracts and ethanol precipitates. The ultimate goal is to optimize the extraction conditions to get the desired GGMs at high yield and purity with as high as possible molar mass for coatings and films packaging like applications.

2 Materials and methods

2.1 Wood material

The *P. halepensis* tree was felled in April 2018 from the forest of Zemmouri's Sahel located in the wilaya (province) of Boumerdes, municipalities of Zemmouri El Bahri and Legata. The Zemmouri's Sahel forest stretches over an area of about 1000 ha and a length of 12 km. It is bounded on the north by the Mediterranean Sea. It is situated between the longitudes 3°37′ and 3°42′ E and the latitudes 36° 44′ and 36°47′ N. The height of the tree was about 22 m and the stem diameter at ground level was about 27 cm.

Wood samples were prepared from a cross sectional sample of an about 6 cm thick disc collected from the stem at about 2.5 m height above the ground. The knots and bark were removed. The disc was then split with a chisel to separate sapwood from heartwood. The sapwood samples were manually chipped to 2 x 1.5 x 0.3 cm size matches. Wood chips were freeze-dried during 72 h and milled separately using a Microfine Grinding Mill equipped with a 1 mm sieve. Ground wood was freeze-dried again for 24 h in order to ensure total drying of the samples. The fraction of 0.5–0.25 mm obtained after fractionation on sieves was used for the study. The samples were stored in airtight plastic bags in a freezer at –18 °C in the dark.

2.2 Extraction with hot-water

Pressurised hot-water extraction of the dried samples was performed with a Dionex Corp, ASE300 (Accelerated Solvent Extractor) according to Willför et al. (2003). Approximately 7 g of wood sample was weighed and extracted at three temperatures (160, 170, and 180 °C) and five extraction times (between 5 and 90 min). The flow rate was steady and the pressure in the extraction cell was 10 MPa. After the extraction has ended, 60% of total water used for the extraction was additionally used for rinsing following the standard setting of ASE extractions of ground wood. After rinsing, the rinsing water was purged from the cell with N_2 for 100 s. The end-pH and volume of all extract solutions were measured shortly after the extraction at room temperature. All water extracts were stored in the dark in closed test tubes at 4 °C.

2.3 Isolation of GGMs by precipitation in ethanol

The optimal precipitation conditions to isolate high-molar-mass GGMs from water extracts were determined. Polysaccharides were precipitated by addition of water extracts to ethanol in order to obtain a water ethanol ratio of 15:85 by volume. The suspensions were left to stand overnight. After decantation of the supernatants, the precipitates were filtered and washed with ethanol, acetone and methyl tert-butyl ether (MTBE), and finally vacuum-dried. The dried precipitates were white powders and stored at 4 °C in the dark. Samples of each precipitate (typically 10 mg) were weighed and dissolved in 5 mL distilled water. Aliquots of sample solutions were taken for different analyses.

2.4 Chemical analyses of extracts

2.4.1 Total dissolved solids (TDS) and end-pH: Total dissolved solids (TDS) were determined gravimetrically after freeze-drying to a constant weight from 2 mL of each aliquot of extract solution. The volume and end-pH were measured at room temperature shortly just after the extraction.

2.4.2 Average molar mass: Determination of weight average molar masses (M_w) was performed by high-performance size exclusion chromatography (HPSEC) equipped with a multiangle laser light scattering (MALLS) detector (mini DAWN, Wyatt Technology, Santa Barbara, CA, USA) and a refractive index (RI) detector (Shimadzu Corporation, Japan). The system consisted of a guard column (Ultrahydrogel 6 × 40 mm, Waters, Milford, MA, USA) and two columns (2 × Ultra-hydrogel™ linear 7.8 × 300 mm, Waters, Milford, MA, USA) connected in series. The water extracts were filtered through a 0.22 µm nylon syringe filter before injection. 0.1 M sodium nitrate solution was used as eluent. The flow rate was 0.5 mL/min and the injection volume was 100 µL. The average molar masses were calculated using Astra software (Wyatt Technology). A dn/dc value of 0.15 mL/g for GGMs was used for the calculations (Brandrup et al. 1999).

2.4.3 Total non-cellulosic carbohydrates: Total non-cellulosic carbohydrates (Mainly GGMs, xylans and pectins) were determined by GC after acid methanolysis according to Sundberg et al. (2009). The main objective was to determine sugar units from non-cellulosic carbohydrates, but also from mono- and oligosaccharides. Non-cellulosic carbohydrates in water extracts and ethanol precipitates were determined by GC of an aliquot of the extract solutions after freeze-drying and silylation of the dry residue.

2.4.4 Monosaccharides and their derivatives: Two milliliter of water extracts were freeze-dried and silylated. The monosaccharides were determined by gas chromatography with flame-ionization detection (GC-FID) on a 25 m × 0.2 mm i.d. column coated with cross linked methyl polysiloxane (HP-1) according to Örså and Holmbom (1994). All of the results were calculated on an oven-dried wood basis.

2.4.5 Acetic acid: Analysis of free acetic acid in the water extracts released during ASE extraction, was carried out by high-performance liquid chromatography (HPLC) with a Synergi Hydro-RP 80R HPLC Column (250 × 4.6 mm, Phenomenex®, Torrance, CA, USA) and an RI detector (Shimadzu, Tokyo, Japan). The pH of the water extract was adjusted to 2.7–2.9 with 30% ortho-phosphoric acid and the eluent contained 20 mM KH_2PO_4 in pure water and pH was adjusted to 2.7–2.9 with ortho-phosphoric acid. The eluent was filtered with a 0.1 µm filter (Anodisc 47, Whatman International, Maidstone, UK) with an injection volume of 100 µL.

For analysis of esterified acetic acid in hemicelluloses, acetyl groups in dissolved GGMs were hydrolyzed by alkaline treatment of extract solutions at 70 °C for 3 h, after adjusting the pH to 12 with 1 M NaOH. After treatment, the pH was re-adjusted to 2.7–2.9 with ortho-phosphoric acid. Hydrolyzed acetic acid was determined by HPLC and an RI detector as described above.

2.4.6 Water-soluble lignin: Lignin in water extracts was measured by UV absorption at the wavelength 280 nm after dilution in water according to the method of Rautiainen and Alén (2010).

3 Results and discussion

3.1 Analyses of water extracts

3.1.1 TDS and pH of water extracts

As expected, the TDS obtained from water extracts increased with the temperature and extraction time (Figure 1). The extraction profiles with different temperatures were very similar. The highest yield of TDS of approximately 182 mg g^{-1} was obtained at 180 °C/40 min, but beyond this concentration, the yield decreased to approximately 171 mg g^{-1}. This degradation was a result of reactions of saccharides including depolymerization, deacetylation, and further degradation of monosaccharides which became dominating at 180 °C after 40 min.

The pH decreased gradually for all the extractions mainly because of the release of acetic acid from acid hydrolysis of acetyl groups associated with polysaccharides (Figure 2). The lower pH level might also partly be caused by the uronic acids released from pectins and xylans into the water phase as suggested by Pranovich et al. (2015). The decrease is more significant at higher temperatures. The

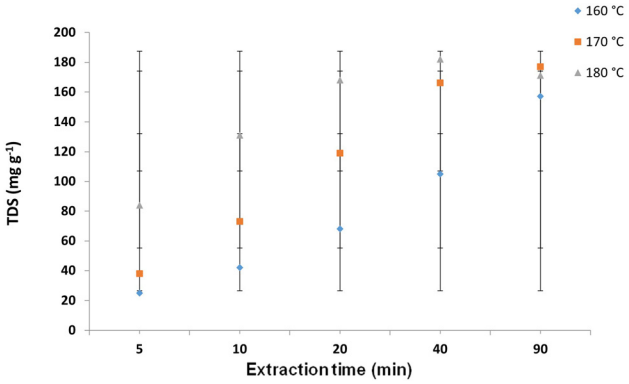

Figure 1: Total dissolved solids (TDS) of water extracts obtained at different temperatures (mg g^{-1} of wood).

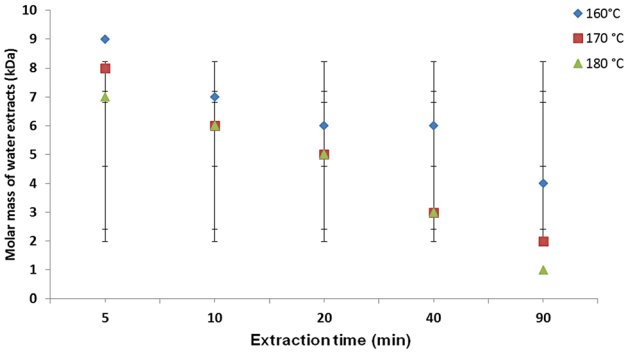

Figure 3: Molar mass of water extracts obtained at different temperatures (kDa).

starting pH at 180 °C dropped from 3.9 to the lowest level of around 3.1 after 90 min extraction time.

TDS for GGMs of spruce ground wood gave a maximum of approximately 250 mg g^{-1} at 180 °C after 60 min (Pranovich et al. 2015). The pH level gradually decreased with extraction time to 3.6–3.8 at 180 °C after 60 min. Extraction at 180 °C of GGMs from spruce resulted in clearly higher TDS and lower pH than GGMs from pine at the same conditions.

3.1.2 Average molar mass of water extracts

The average molar masses (M_w) of the water extracts are shown in Figure 3. The average M_w decreased as the temperature increased and the highest Mw, 9 kDa, was determined at 160 °C after 5 min. The lowest M_w, 1 kDa, was determined at 180 °C after 90 min, i.e., under the most severe conditions applied, where the hydrolysis is most extensive.

Extraction of GGMs from spruce at 160 °C/5 min gave the highest M_w, being 35 kDa (Song et al. 2008) much higher than the M_w obtained for GGMs from pine at the same conditions.

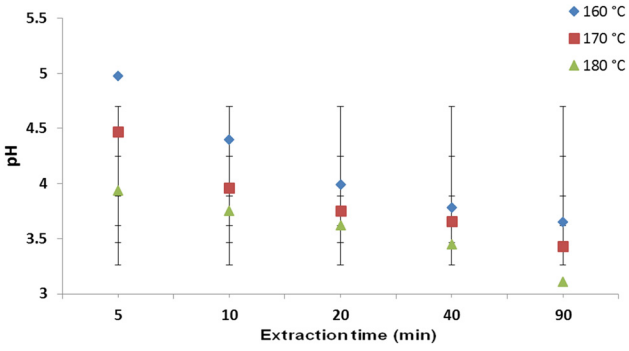

Figure 2: pH of water extracts obtained at different temperatures.

The pH profile during extraction should be optimized to minimize the hydrolysis of acetyl groups and the hydrolytic cleavage of GGM chains with the objective to obtain a high yield with a reasonably high molar mass (Pranovich et al. 2015).

3.1.3 Non-cellulosic carbohydrates of water extracts

The yield of 755–767 mg g^{-1} of hemicelluloses and pectins (Rha+GalA) were extracted from ground wood at 160–180 °C (Figure 4). The highest yield was obtained at 180 °C/40 min amounting to about 767 mg g^{-1}. This yield dropped clearly after 90 min and all sugar components decreased in varying proportions except glucose (Glc) which increased slightly from 79.6 to 95.7 mg g^{-1}. The decrease was most significant for xylose (Xyl) (160 to 58 mg g^{-1}) and mannose (Man) (339 to 286 mg g^{-1}).

Hemicelluloses and pectins are amorphous. During hot-water extraction, the glycosidic bonds are partly hydrolyzed involving the breakdown of the long chains hemicellulose and pectins leading to the formation of shorter chained oligomers and then monosaccharides (Lai 2001; Leppänen et al. 2011). The yield of monosaccharides then formed increased at elevated temperatures during extended extraction times. GGM impurities with other hemicelluloses dominated in all extracts even at the most severe conditions (Figure 4).

Arabinose was the dominating monosaccharide reaching a maximum and thereafter, decreased from 5 min extraction time (Figure 5). By increasing extraction temperature to 180 °C, the amount of arabinose decreased much faster from the highest amount of 89.2 mg g^{-1} to the smallest amount of 9.5 mg g^{-1} after 90 min. Conversion of arabinose, involving acid-catalyzed degradation reactions

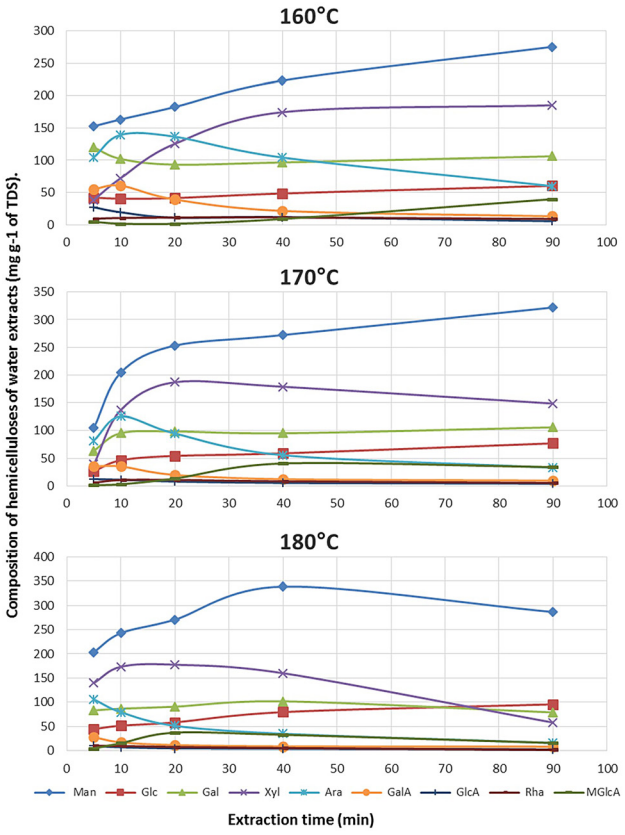

Figure 4: Amounts and composition of hemicelluloses of water extracts obtained at different temperatures (mg g^{-1} of TDS).

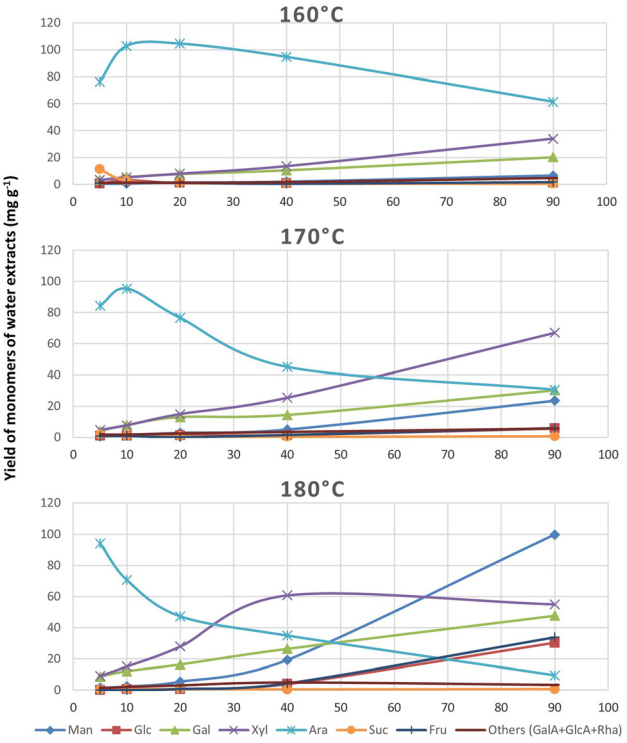

Figure 5: Amounts of monomers of water extracts obtained at different temperatures (mg g^{-1} of TDS).

was suggested to lead to the formation of oxygenate molecules such as furfural (Lai 2001).

Xylose, was the second most abundant monosaccharide in the water extracts, exhibited a growing trend along with time. At 180 °C, the xylose kept the same trend reaching the largest amount of 65 mg g^{-1} the end of extraction.

Mannose was the principal hexose especially after the extended extraction times. During 180 °C extraction, the amount of mannose was very small in the beginning amounting to 1.6 mg g^{-1}, increased strongly to its maximum of 49.8 mg g^{-1} after 90 min (Figure 5).

Most of sugar units in hemicellulose are linked together by β-1,4-glycosidic bonds. When galactose is present as a side chain linked to the main chain, the polymeric backbone is called galactomannan, and when both glucose and galactose are linked to the mannose sugars of the main chain, it is called galactoglucomannan (GGMs). The amount of galactose in the extracts was higher than that of mannose until 180 °C/90 min. This indicates that galactose side substituents were cleaved by hydrolysis much faster than the main glucomannan chain.

Glucose increased slightly during 160 and 170 °C hot-water extractions but increased dramatically to reach 30.3 mg g^{-1}, at 180 °C/90 min. GGMs is the main source for glucose increased at higher temperatures after extended extraction times. Glucose is also present in pine wood in a free form, which is easily dissolved in water and easily hydrolyzed by acid reactions.

Fructose was found in small amounts and a notable amount of rhamnose was found after the extended extraction probably originated from pectins.

Only trace amounts of GalA and GlcA were present in the extracts, may be because of their extensive degradation or to slow hydrolytic cleavage of the uronic acid glycosidic bonds.

3.1.4 Acetic acid of water extracts

The amount of free acetic acid released from GGMs exhibited a growing trend during 160 °C hot-water extraction, reaching the amount of 1.3 mg g^{-1} after 90 min extraction time (Figure 6). However, at the time point of 90 min, during the most severe condition of 180 °C, the corresponding ratio increased dramatically reaching 3.6 mg g^{-1}. This increase was due essentially to acetyl

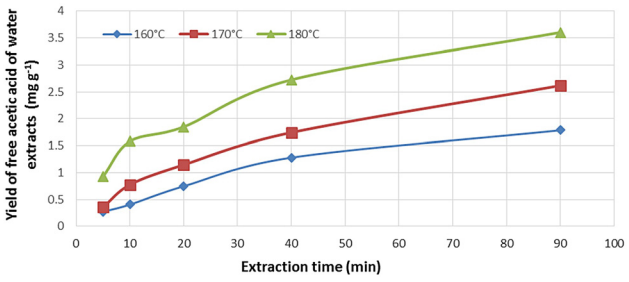

Figure 6: Amounts of free acetic acid of water extracts released during ASE extraction at different temperatures (mg g^{-1} of wood).

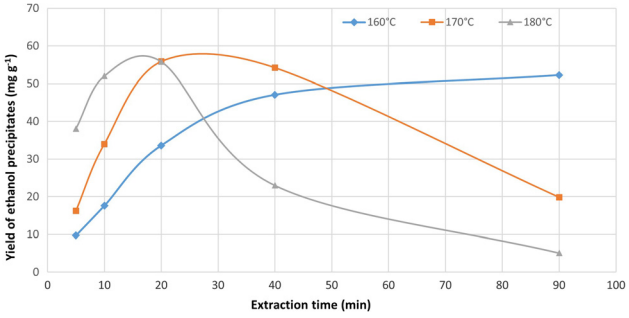

Figure 8: Amounts of precipitates in ethanol from extracts obtained at different temperatures (mg g^{-1} of wood).

groups, which were hydrolyzed from the dissolved GGMs at the most sever conditions.

3.1.5 Lignin of water extracts

Lignin in the water extracts released during the extraction of ground sapwood was determined by UV-absorption measurement. During hot-water extraction, more lignin was released at higher temperatures and longer extraction times (Figure 7). At sever extraction conditions of temperature, 180 °C and after 90 min extraction time, the released lignin increased from 13.6 mg g^{-1} to the highest yield of about 247 mg g^{-1}.

3.2 Analyses of ethanol precipitates

3.2.1 Amounts of ethanol precipitates

In order to explore the possibilities to obtain GGM in high yield from pine sapwood and to gain deeper understanding of the chemical composition and the extraction process involved, the water extracts were subjected to ethanol precipitation as described above. The largest amounts were obtained at 160 °C/90 min, 170 °C/20 min, and 180 °C/20 min accounting for 52.2, 55.8, and 56.2 mg g^{-1}, respectively, whereas the smallest amounts were obtained at 160 °C/5 min, 170 °C/5 min, and 180 °C/90 min accounting for 9.4, 15.7, and 5.0 mg g^{-1}, respectively (Figure 8).

At the most sever conditions of temperature, the profile of ethanol precipitates during 180 °C extraction exhibited the opposite pattern, which precipitates in ethanol increased much faster within the range of 5–20 min, but decreased considerably at extended extraction times reaching the lowest yield after 90 min.

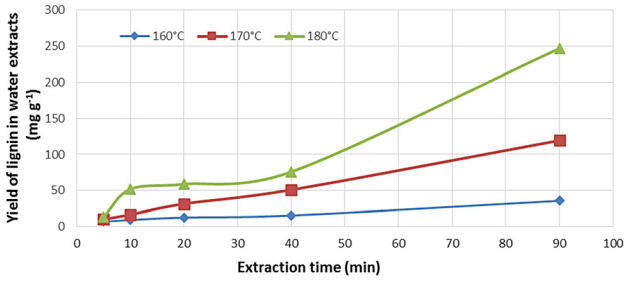

Figure 7: Amounts of lignin in water extracts released during ASE extraction at different temperatures (mg g^{-1} of wood).

3.2.2 Molar mass of precipitates

The molar masses (M_w) of precipitates in ethanol obtained by HPSEC were dependent strongly on the temperature and extraction time, where longer retention time corresponds to lower molar mass (Figure 9).

During 160 °C extraction, the M_w of hemicelluloses and pectins extracts in the ethanol precipitates decreased by 5 times, from 27 to 5 kDa at the end of extraction. The extracts obtained during the 170 °C extraction exhibited lower Mw compared to 160 °C extraction which, the average M_w value decreased from 15 kDa to about 4 kDa after 90 min. At higher temperatures and extended extraction times, the M_w of hemicelluloses and pectins in the ethanol precipitates became less dependent on the extraction time. As shown in Figure 9, the M_w of the ethanol precipitates during the 180 °C hot-extraction was ranged between 4 and 8 kDa. The M_w values of these ethanol precipitates were much lower compared to 160 °C and 170 °C extractions because most of the high-molar-mass compounds were already removed from the wood at earlier stages of extraction and probably because the intensive hydrolytic degradation of hemicelluloses and pectins.

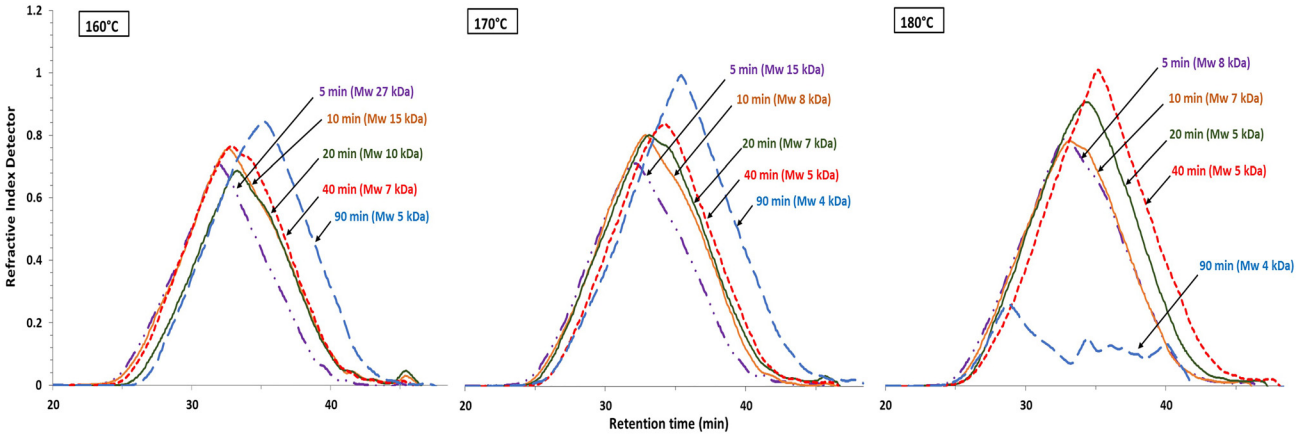

Figure 9: HPSEC of precipitates at different temperatures. Calculated weight average M_w values given in parenthesis.

3.2.3 Non-cellulosic carbohydrates

The hemicelluloses and pectins (determined as anhydrosugars) obtained from precipitation in ethanol had a similar sugar unit composition in all extractions. However, the corresponding ratios were quite different and the M_w varied following high polymeric, low polymeric and oligomeric states (Figure 10).

The total yield of hemicelluloses and pectins increased during the 160 °C extraction reaching the maximum of 927 mg g^{-1} at the end of extraction (Figure 10). In the contract, during the 170 °C extraction, the total yield increased to its maximum 903.4 mg g^{-1} at 10 min, but decreased slightly reaching 773.8 mg g^{-1} at the end of extraction. The decrease was much faster and more significant during the 180 °C extraction dropped from 864.6 mg g^{-1} at 10 min to reach the minimum yield of 434.5 mg g^{-1} at the end of extraction time.

GGM-derived mannose, glucose, and galactose units were the main polysaccharide dominating in all ethanol precipitates (Figure 11). The total yield of GGMs reached the maximum at 160 °C/90 min, 170 °C/90 min, and 180 °C/20 min, amounting 720, 700, and 639 mg g^{-1}, respectively.

At the more severe conditions by increasing temperature and consequently decreasing molar mass during the third extraction, the portion of mannose, glucose, and galactose decreased significantly reaching the minimum of 345 mg g^{-1} at the end of the extraction.

GGMs were precipitated in larger amounts than arabino-4-*O*-methylglucuronoxylans (xylans), probably because their higher molar mass involved more stability against acid hydrolysis. The content of xylans, shown in Figure 12, increased from the beginning of the 160 °C (103.5 mg g^{-1}) reaching a maximum after 40 min (334.4 mg g^{-1}), and then decreased along with the extended extraction time to 268.2 mg g^{-1} of precipitate after 90 min. The xylan content increased faster from the beginning of the 170 °C extraction (165 mg g^{-1}) reaching a maximum after 20 min (318.8 mg g^{-1}), and then decreased to 156.7 mg g^{-1} after 90 min. At the beginning of the 180 °C extraction, the xylan content was

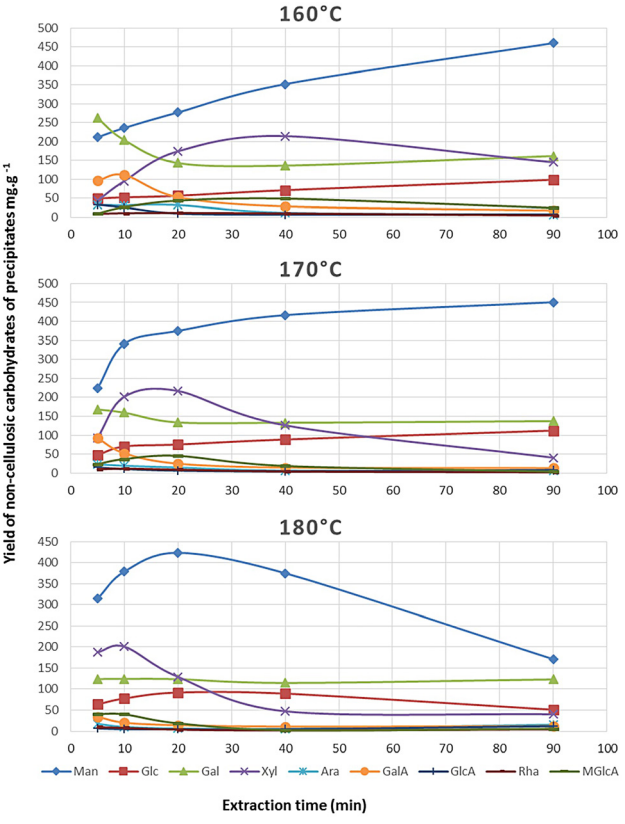

Figure 10: Non-cellulosic carbohydrates of precipitates obtained from extracts at different temperatures (mg g^{-1} of ethanol precipitates).

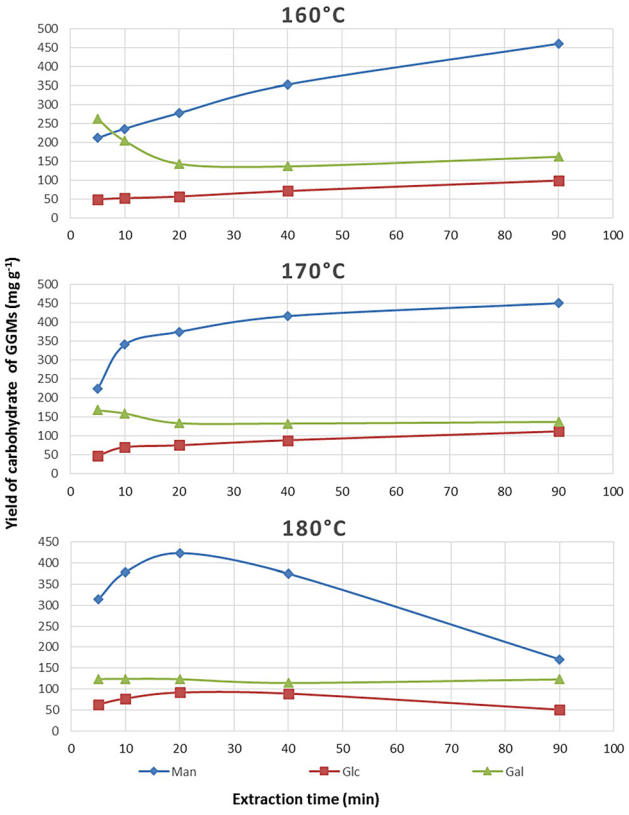

Figure 11: Carbohydrate composition of GGMs obtained at different temperatures (mg g^{-1} of ethanol precipitates).

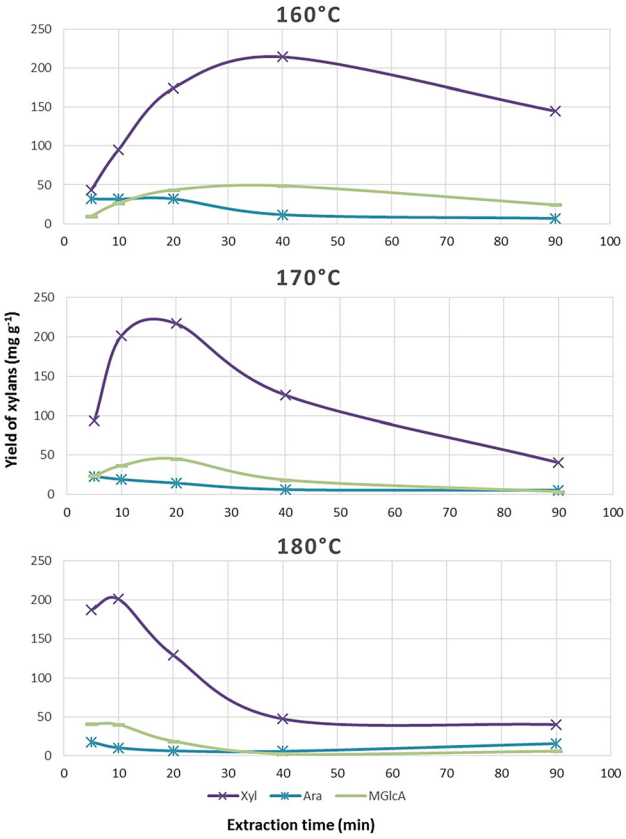

Figure 12: Carbohydrate composition of xylans at different temperatures (mg g^{-1} of ethanol precipitates).

found to be higher, reaching a maximum after 10 min (292 mg g^{-1}), but already decreased much more to the minimum level content after 90 min (96.7 mg g^{-1}). The continuous growth in GGM trends was more stable as compared to xylans, because the polymeric chains of GGMs during hot-water extraction are more stable than xylans chains.

The content of pectins in the ethanol precipitates (Figure 13) from the 160 °C extraction had a maximum after 10 min (122 mg g^{-1} of precipitate), but decreased thereafter dramatically (22 mg g^{-1}). Pectins contents from the 170 and 180 °C extractions were slightly lower than that from the 160 °C extraction. The corresponding contents reached a maximum yield of 102 mg g^{-1} at 170 °C/5 min and 44 mg g^{-1} at 180 °C/5 min, decreased thereafter to the minimum yield of 15 mg g^{-1} at 170 °C/90 min and 11.3 mg g^{-1} at 180 °C/90 min.

3.2.4 Acetic acid of precipitates

The amount of release acetic acid after alkaline hydrolysis of precipitated hemicelluloses (Figure 14) increased to the maximum at 160 °C/40 min, 170 °C/40 min and 180 °C/20 min, amounting 23.1, 19.7, and 22.3 mg g^{-1} respectively. At the extended extraction times, the amount of acetic acid decreased significantly reaching the amount of 19.1, 13.8, and 13.5 mg g^{-1}, respectively at the end of extraction time. This increase was due essentially to acetyl groups, which were hydrolyzed from the dissolved GGMs.

3.3 Preparative extraction of GGMs at 170 °C, 20 min

P. halepensis sapwood was extracted with pressurized hot-water at 160–180 °C through five extraction times ranged between 5 and 90 min. Only small amounts of hemicelluloses and pectins were obtained at the beginning of each hot-water extraction, but extraction process was significantly enhanced when extended times and higher extraction temperatures were applied. The changes on temperature and time parameters during hot-water extraction were shown to affect the molar-mass characteristics and yield of GGMs. A good compromise between these two parameters was chosen in order to optimize the extraction efficiency of GGMs and their corresponding

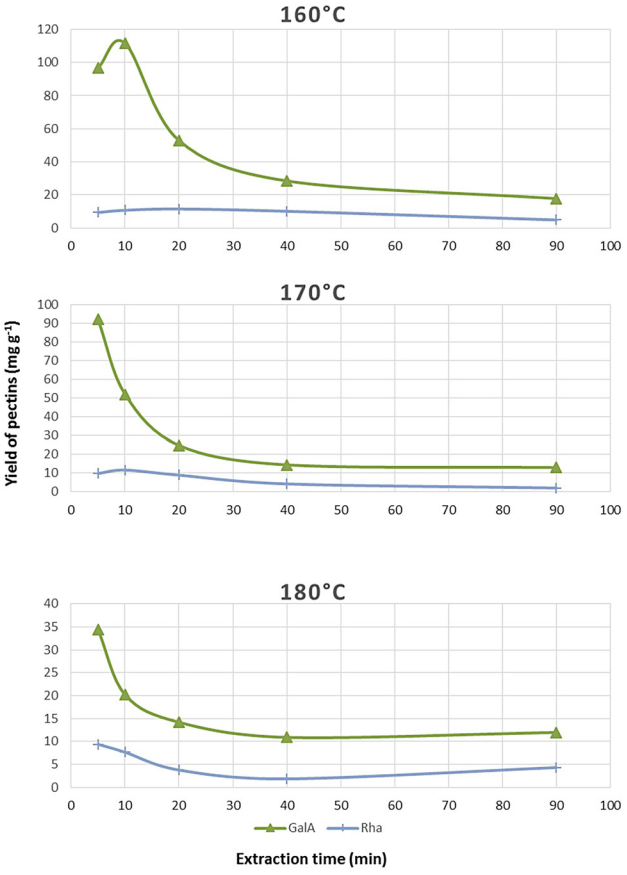

Figure 13: Carbohydrate composition of pectins obtained at different temperatures (mg g^{-1} of ethanol precipitates).

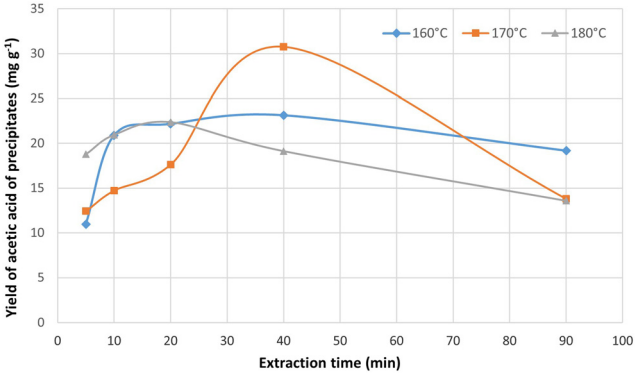

Figure 14: Acetic acid from alkaline hydrolysis of precipitates obtained from water extracts at different temperatures (mg g^{-1} of ethanol precipitates).

chemical and structural characteristics for coatings or films packaging like applications.

Optimal extraction parameters of GGMs were identified during the second hot-water extraction at 170 °C after 20 min extraction time, with an average M_w component of 7 kDa leading to GGMs yield of approximately 56 mg $g^{-1}_{o.d.m}$, corresponding to about 6% on dry wood basis.

3.3.1 Non-cellulosic carbohydrates

The carbohydrate composition of hemicelluloses and pectins is shown in Figure 15. GGMs were the first major hemicelluloses, noticed in Figure 15 by large amounts of mannose (Man), glucose (Glc), and galactose (Gal) accounting approximatively 629.6 mg g^{-1}. Xylans are the second most abundant represented by xylose (Xyl), arabinose (Ara) and 4-O-methylglucuronic acid (MeGlcA) accounting approximatively 291 mg g^{-1}. Pectins were present in small amounts accounting approximatively 44.2 mg g^{-1}.

3.3.2 Analysis of ethanol soluble substance

The chemical composition of ethanol soluble substance after precipitation of GGMs is shown in Figure 16. Arabinose (Ara) was the major monomer accounting approximatively 182 mg g^{-1}. Xylose (Xyl) and galactose (Gal) were present in small amounts accounting approximately 23 and 20.3 mg g^{-1}.

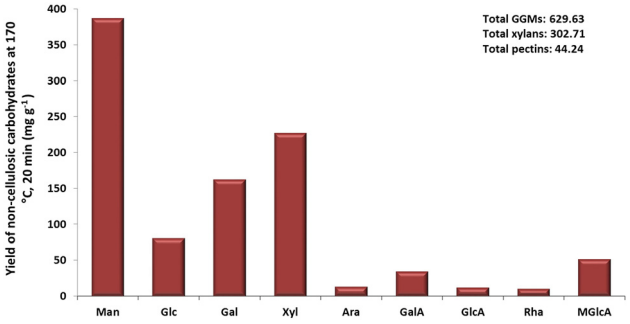

Figure 15: Non-cellulosic carbohydrates obtained at 170 °C, 20 min (mg g^{-1} of ethanol precipitates).

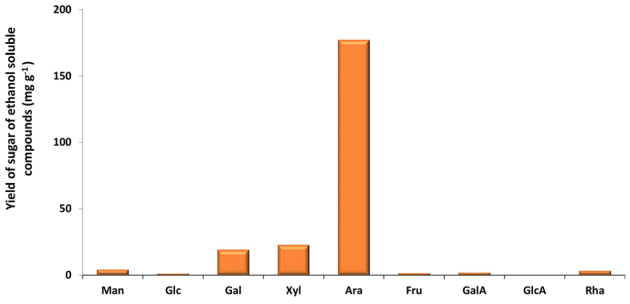

Figure 16: Sugar composition of ethanol soluble compounds from water extracts (mg g^{-1} of wood).

4 Conclusion

The main objective of this work was to study the extraction conditions which allowed to obtain specific GGMs at high yield and purity with as high as possible molar mass for coatings or films packaging like applications. The influence of different experimental parameters (extraction time and temperature) on the effectiveness of GGMs extraction processes was studied. GGM yields of ground wood from pine were obtained via hot-water extraction at three temperatures in the range 160–180 °C with five extraction times ranged between 5 and 90 min in order to optimize extraction parameters. The pH decreased gradually for all the extractions because of the release of acetic acid from acid hydrolysis of acetyl groups associated with polysaccharides.

The highest yield of TDS was obtained at 180 °C and 40 min extraction time, but decreased significantly because of the reactions of saccharides including depolymerization, deacetylation, and further degradation of monosaccharides. The average M_w decreased as the temperature increased and the highest M_w was determined at 160 °C after 5 min, while the lowest M_w was determined at 180 °C after 90 min. The highest yield of hemicelluloses and pectins (Rha+GalA) was obtained at 180 °C after 40 min time. This yield dropped clearly for 90 min extraction time and all sugar components decreased in varying proportions except glucose (Glc) which increased slightly. The decrease was most significant for xylose (Xyl) and mannose (Man). The amount of free acetic acid released from GGMs increased dramatically during the most severe condition of 180 °C, essentially due to acetyl groups, which were hydrolyzed from the dissolved GGMs. Lignin was released at higher temperatures and longer extraction times increased at sever extraction conditions of temperature.

The M_w of ethanol precipitates were dependent strongly on the temperature and extraction time, which longer retention time corresponds to lower molar mass. The hemicelluloses and pectins from precipitation in ethanol had a similar sugar unit composition in all extractions. However, the corresponding ratios were quite different and the M_w varied following high polymeric, low polymeric and oligomeric states. The amount of free acetic acid increased from the beginning and then decreased. This increase was due essentially to acetyl groups, which were hydrolyzed from the dissolved GGMs.

A good compromise between temperature and time parameters during hot-water extraction was chosen in order to optimize the extraction efficiency of GGMs and their corresponding chemical and structural characteristics for biofilms and hydrogels applications. Optimal extraction parameters of GGMs were identified during the 170 °C hot-water extraction after 20 min extraction time, with an average M_w component of 7 kDa leading to GGMs yield of approximately 56 mg $g^{-1}_{o.d.m}$, corresponding to 6% on dry wood basis.

Acknowledgments: This work was part of the doctoral research activities of ((insert author name/s)) at the Johan Gadolin Process Chemistry Centre (PCC) at Åbo Akademi University, Finland. We thank The Algerian Ministry of Higher Education and Scientific Research for funding support within "Le Projet National Exceptionnel (P.N.E)".
Author contribution: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

References

Ebringerova, A., Hromadkova, Z., Kacurakova, M., and Antal, M. (1994). Quaternized xylans: synthesis and structural characterization. Carbohydr. Polym. 24: 301–308, ISSN 0144-8617.

Ebringerova, A., Hromadkova, Z., and Heinze, T. (2005). Hemicellulose. Adv. Polym. Sci. 186: 1–67.

Gabrielii, I., Gatenholm, P., Glasser, W., Jain, R., and Kenne, L. (2000). Separation characterization and hydrogel formation of hemicellulose from aspen wood. Carbohydr. Polym. 43: 367–374.

Gonzalez, R., Jameel, H., Chang, H., Treasure, T., Pirraglia, A., and Saloni, D. (2011). Thermo-mechanical pulping as a pretreatment for agricultural biomass as biochemical conversion. BioResources 6: 1599–1614.

Hartman, J., Albertsson, A., Lindblad, M., and Sjöberg, J. (2006) Oxygen barrier materials from renewable sources: material properties of softwood hemicellulose-based films. Appl. Polym., https://doi.org/10.1002/app.22958.

Hosseinaei, O., Wang, T., Rials, G., Xing, C., and Zhang, Y. (2011). Effects of decreasing carbohydrate content of wood strand on their properties. Cellulose 18: 841–850.

Kisonen, V., Prakobna, K., Xu, C., Salminen, A., Mikkonen, K., Valtakari, D., Eklund, P., Seppälä, J., Tenkanen, M., and Willför, S. (2015). Composite films of nanofibrillated cellulose and O-acetyl galactoglucomannan (GGM) coated with succinic esters of GGM showing potential as barrier material in food packaging. J. Mater. Sci. 50: 3189–3199.

Lai, Y.Z. (2001). *Chemical degradation in wood and cellulosic chemistry*, 2nd ed. Marcel Dekker, Inc., pp. 443–512.

Laine, C. (2005). *Structures of hemicellulose and pectins in wood and pulp*. Finland, Espoo: Helsinki University of Technology, Oy Keskuslaboratorio. Department of Chemical Technology KCL.

Leppänen, K., Spetz, P., Pranovich, A., Hartonen, K., Kitunen, V., and Ilvesniemi, H. (2011). Pressurised hot-water extraction of Norway spruce hemicelluloses using flow-through system. Wood Sci. Technol. 45: 223–236.

Brandrup, J., Immergut, E.H., and Grulke, E.A. (1999). *Polymer handbook*, 4th ed. New York: Wiley, pp. 547–627.

Mikkonen, K.S., Tenkanen, M., Cooke, P., Xu, C., Rita, H., Willför, S., Holmbom, B., Hicks, K.B., and Yadav, M.P. (2009). Mannans as stabilizers of oil-in-water beverage emulsions. Food Sci. Technol. 42: 849–855.

Mikkonen, K.S., Mari, I., Heikkilä, M., Willför, S., and Tenkanen, M. (2012). Films from glyoxal-crosslinked spruce galactoglucomannans plasticized with sorbitol. Int. J. Polym. Sci. 482810, https://doi.org/10.1155/2012/482810.

Mikkonen, K.S., Stevanic, J.S., and Joly, C. (2011). Composite films from spruce galactoglucomannans with microfibrillated spruce wood cellulose. Cellulose 18: 713–726.

Örsä, F. and Holmbom, B. (1994). A convenient method for determination of wood extractives in paper-making process waters and effluents. J. Pulp Pap. Sci. 20: J361–366.

Pranovich, A., Holmbom, B., and Willför, S. (2015). Two-stage hot-water extraction of galactoglucomannans from spruce wood. J. Wood Chem. Technol. 36: 140–156.

Rana, V., Rai, P., Tiwary, A.K., NSingh, R.S., Kennedy, J.F., and Knill, C.J. (2011). Modified gums: approaches and applications in drug delivery. Carbohydr. Polym. 83: 1031–1047.

Rautiainen, R. and Alén, R. (2010). Characterization of black liquors from kraft pulping of first thinning Scots pine (*Pinus sylvestris*). Holzforschung 64: 7–12.

Shevchenko, S.M., Chang, K., Robinson, J., and Saddler, J.N. (2000). Optimization of monosaccharide recovery by post-hydrolysis of the water-soluble hemicellulose component after steam explosion of softwood chips. Bioresour. Technol. 72: 207–211.

Sjöström, E. (1993). *Wood chemistry: fundamentals and application*. London: Academic Press.

Sjöström, E., and Alén, R. (1998). *Analytical methods in wood chemistry, pulping, and papermaking*. Berlin: Springer Series in Wood Science, Springer.

Song, T., Pranovich, A., Sumerskiy, I., and Holmbom, B. (2008). Extraction of galactoglucomannan from spruce wood with pressurised hot-water. Holzforschung 62: 659–666.

Sundberg, A., Strand, A., Vähäsalo, L., and Holmbom, B. (2009). Phase distribution of resin and fatty acids in colloidal wood pitch emulsions at different pH-levels. J. Dispersion Sci. Technol. 30: 912–919.

Trovatti, E., Fernandes, S., and Rubatat, L. (2012). Pullulan–nanofibrillated cellulose composite films with improved thermal and mechanical properties. Compos. Sci. Technol. 72: 1556–1561.

Willför, S., Hemming, J., Reunanen, M., Eckerman, C., and Holmbom, B. (2003). Lignans and lipophilic extractives in Norway spruce knots and stemwood. Holzforschung 57: 7–36.

Willför, S., Sundberg, A., Hemming, J., and Holmbom, B. (2005). Polysaccharides in some industrially important softwood species. Polysaccharides in some. Wood Sci. Technol. 39: 245–257.

Willfor, S., Sundberg, K., Tenkanen, M., and Holmbom, B. (2008). Spruce-derived mannans: a potential raw material for hydrocolloids and novel advanced natural materials. Carbohydr. Polym. 72: 197–210.

Xu, C., Willför, S., and Holmbom, B. (2008). Rheological properties of mixtures of spruce galactoglucomannans and Konjak glucomannans or some other polysaccharides. BioResources 3: 713–730.

Xu, C., Pranovich, A., Hemming, J., Holmbom, B., Albrecht, S., Schols, H. A., and Willför, S. (2009). Hydrolytic stability of water-soluble spruce *O*-acetyl galactoglucomannans. Holzforschung 63: 61–68.

Zhou, C., Beltramini, J., Fan, Y., and Lu, G. (2006). Chemoselective catalytic conversion of glycerol as a biorenewable source to valuable commodity chemicals. Chem. Soc. Rev. 37: 527–549.