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Heat Extraction of Corn Fiber Hemicellulose

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Abstract

Water-soluble hemicellulose was extracted from corn fiber with microwave-assisted heat treatment. The effects of treatment temperature and initial pH of the aqueous extraction media were investigated regarding hemicellulose recovery and molecular mass of the isolated polysaccharides. In treatments carried out at neutral pH (simple water extraction), it has been demonstrated that hemicellulose recovery could be increased by applying higher treatment temperatures. However, the molecular weight of isolated hemicellulose gets significantly lower. For example, 10% of the raw materials' xylan was extracted at 160°C and about 30% recovery was reached at 210°C. However, the molecular mass of the isolated polysaccharide at 210°C (5.82×10^4) was about half of that measured at 160°C (1.37×10^5). Reducing the pH with sulfuric acid resulted in shorter polymer chains (1.7×10^4) and lower hemicellulose yields (2.2%). Application of sodium hydroxide in the treatment showed that, compared with acid, considerably higher yields (11%) with longer polysaccharide chains (1.3×10^5) could be obtained.

Index Entries: Alkaline extraction; carbohydrate analysis; microwave-assisted fractionation; size exclusion chromatography; weight-average molecular weight; maize.

Introduction

Xylose-rich, water-soluble hemicelluloses have potential applications in numerous industries. They are used as ingredients in functional foods produced in Japan *(1)*, and it is suggested that agriculture might be another target area of utilization as "growth factor-like" properties have been shown *(2)*. Furthermore, using these renewable materials in the polymer industry for the production of biodegradable plastics provides a new

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possibility to develop environmentally sound technologies and products *(3)*. Xylan-based dietary fibers *(4)* can lower blood cholesterol level *(5)*, reduce stomach ulcer lesions *(6)*, stimulate the growth of intestinal Bifidobacteria *(7)*, and xylans might act as human immunodeficiency virus inhibitor as well *(8)*. Hemicelluloses have acceptable odor, low-caloric values, and are noncarcinogenic; thus, as filler substances they could be part of novel, fortified, and specialty foods as well, which are tools of antiobesity diets and enteral nutrition. Finally, these polymers could be constituents of synbiotics consisting of both a prebiotic and a live microbial food ingredient called probiotics *(9)*.

There are two main factors determining the practical use of hemicellulose-originated polysaccharide isolates. On one hand, the chemical (sugar) composition, which primarily is a plant specific feature, needs to be considered. Corn fiber hemicellulose mainly consists of xylose (48%) and arabinose (35%), and also small amounts of galactose (7%) and glucuronic acid (10%) *(10)*. The structure of corn fiber hemicellulose has been studied extensively. It is a branched arabinoglucuronoxylan in which 4-O-methylglucuronic acid groups, arabinose, and trisaccharide groups made up of arabinose, xylose, and galactose are linked directly to the main xylan backbone *(11)*.

On the other hand, the way of lignocellulosics fractionation influences the polymer structure, thus the possible field of application. There are four generally accepted main fractionation categories: physical, chemical, biological, and physico-chemical methods *(12)*. Heat treatments such as steam explosion, microwave irradiation *(13,14)*, and hot water extraction *(15,16)* are regarded as physico-chemical treatments. Chemical treatment is carried out with the aid of bases *(17–19)*, acids, or organic solvents. Lignin removal can be carried out with biological methods such as the use of lignin degrading microbes.

Lately, biofuels have gained great interest because of environmental considerations as well as global increase in cost of fossil fuels. In the production of ethanol from corn the first step is wet-milling, which yields different byproducts, including corn fiber. Generally, this corn fiber is utilized as animal feed. An anticipated increase in ethanol production is expected to saturate the animal feed market. Therefore, it is of great importance to look for alternative applications of corn fiber and to examine the possibilities to upgrade it to value-added products. One possibility would be to separate the different components in the fiber, thereby obtaining refined products for further processing.

In this study, corn fiber hemicellulose was fractionated using microwave irradiation according to the flow sheet shown in Fig. 1. This treatment involves both physical and chemical methods and has previously been applied for the extraction of galactoglucomannan hemicellulose from spruce *(20,21)*. The effect of treatment temperature and pH were investigated at fixed residence time. Hemicellulose recovery, the molecular weight

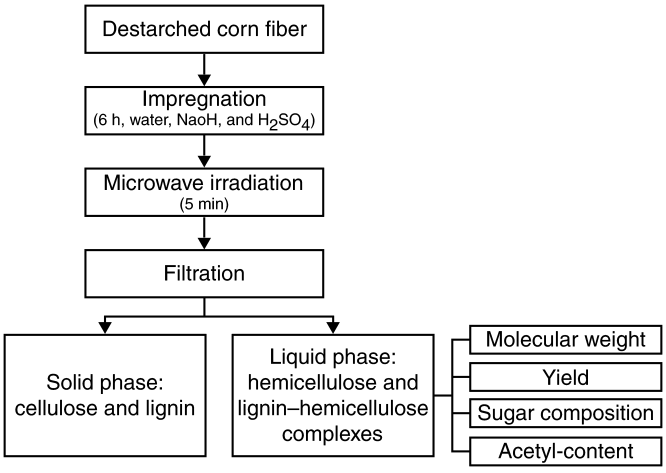

Fig. 1. Schematic flowchart of hemicellulose isolation and characterization from corn fiber.

of solubilized hemicellulose, the sugar composition, and the acetyl group content of isolates obtained under different treatment conditions were determined.

Materials and Methods

Preparation and Analysis of Starch Free Corn Fiber

Corn fiber, which is a byproduct of corn wet-milling starch manufacturing, was kindly provided by Hungrana Co. (Hungary). The average particle size of corn fiber was less than 8 mm and it was not ground before use. Starch was removed in a two-step enzymatic hydrolysis process using thermostable α-amylase (Thermamyl Supra, Novozymes, Denmark) in a 31-L B.Braun DCU-3 laboratory bioreactor (B. Braun Biotech AG, Germany), at 8.7% dry matter content in 0.05 *M* sodium acetate buffer solution (pH 4.8) with continuous stirring at 250 rpm. In the first step 19.4 mL of Thermamyl Supra was added. The mixture was heated up to 120°C and after 20 min the slurry was cooled down to 90°C at this temperature another 19.4 mL of Thermamyl Supra was added and hydrolysis was allowed to continue for another hour. On completion of starch hydrolysis, the slurry was filtered through a 150-mm mesh nylon filter. The liquid fraction was discharged and the filter cake was washed with hot distilled water. The starch free corn fiber (SFCF) was air-dried and stored at room temperature. The dry matter content of air-dried SFCF was 90.9%.

The chemical composition of SFCF was determined with the Hägglund procedure *(22)* followed by high-performance liquid chromatography (HPLC) analysis of the sugars. SFCF contained, given as weight percentages of dried material, 22.1% cellulose, 36.5% hemicellulose, 19.2% lignin, and

22.3% other compounds such as extractives, protein, and ash. On the basis of dry SFCF the hemicellulose fraction contained (expressed in polysaccharide form): 13.7% arabinose, 5.6% galactose, and 17.2% xylose.

Microwave-Assisted Heat Treatment of SFCF

Before heat treatment, 10.0 g air-dried SFCF was soaked with 199.0 g impregnation medium: either distilled water or aqueous solutions of sulfuric acid (0.025% and 0.5%) or sodium hydroxide (0.025% and 0.5%) for 6 h. The whole slurry was transferred into a 350-mL well-sealed Teflon treatment vessel, which was then placed into a microwave oven (Milestone MLS-1200 Mega Microwave Workstation, Sorisole, Italy). The microwave oven was programmed to heat up the reaction mixture in a 2-min cycle to the desired treatment temperature. On reaching the set point, the temperature was controlled at a constant value for another 5 min. The temperature inside the vessel was regulated through a thermocouple immersed into the slurry. Temperatures of 100, 130, 160, 180, 200, and 210°C, respectively were screened. On completion of the heat treatment cycle, the reaction vessel was removed from the oven and cooled down to room temperature with cold water stream. Fibrous residue of the treated SFCF was separated by vacuum filtration of the water-soluble hemicellulose fraction, which was then analyzed for both monomeric sugar and hemicellulose content.

Extraction of Hemicellulose-B

Hemicellulose-B isolation from SFCF was performed according to the procedure described earlier *(23)*. In the first step hemicellulose was extracted from the material by treatment with 2% sodium hydroxide solution at 120°C for 60 min. The dry matter content of the mixture was 10%. After heat treatment, the suspension was rapidly cooled to room temperature and the solid fraction was separated by filtration on a 150-µm mesh nylon filter. The filtrate was collected and the pH of the liquid was adjusted to 4.5 with 35% hydrochloric acid. Double volume of 95% ethanol was added to the filtrate. After 1 d at room temperature the precipitated hemicellulose-B was collected by filtration, rinsed with ethanol, and dried at 105°C. Sugar composition, acetyl group content, and average molecular weight of the isolate were determined as described under "Analysis" for samples obtained in microwave heat treatment.

Analysis

Determination of Hemicellulose Content

Supernatants obtained after heat treatment were directly analyzed for monomeric sugars using high-performance liquid chromatography (HPLC) pulsed amperometric detection (PAD) (described under "Carbohydrate Analysis"). For the determination of hemicellulose polymers a milliliter of

each liquid obtained in the heat treatment was hydrolyzed with an equivalent amount of 0.4 *M* sulfuric acid solution in an autoclave at 121°C for 60 min. Samples were subjected to HPLC analysis and the differences between sugar concentrations measured after and before acid hydrolysis were used to calculate the amount of polysaccharides (correction factor of 1.13 for water on hydrolysis was applied).

Fractionation of Polysaccharides by Size Exclusion Chromatography

Weight-averaged molecular weight (M_w) of hemicellulose extracted from SFCF was determined by size exclusion chromatography (SEC). A 500-µL aliquot was loaded on a two-column configuration containing a Superdex 75 and a Superdex 200 column (GE Healthcare, formerly Amersham Biosciences, Uppsala, Sweden) connected in series to an fast protein liquid chromatography (FPLC) system (GE Healthcare). Elution of samples with ultrapure water at a flow rate of 0.5 mL/min was followed by refractive index (RI) detection (RID, Erma-inc, Tokyo, Japan) and ultraviolet detection at 280 nm (GE Healthcare), enabling the detection of both polysaccharides and ultraviolet absorbing compound such as solubilized lignin degradation products. The molecular weight distribution of polysaccharides was determined using dextran (Fluka Chemie AG, Buchs, Switzerland) calibration standards with M_w of 1270, 5220, 11,600, 23,800, and 48,600, respectively. The total dead volume of the two-column configuration (47 mL) was assessed with acetone and the void volume, using Blue Dextran (Fluka Chemie AG) was determined to be 16 mL. During elution, fractions of 2 mL were collected. Fractions showing RI signal in the chromatograms were subjected to further polysaccharide analysis as described in the hemicellulose Analysis section.

Carbohydrate Analysis

Samples for sugar analysis, obtained during heat treatment or SEC elution, were first filtered through a 0.2-µm syringe filter (Acrodisc, PALL Gelman Laboratory, MI). Sugars were analyzed using high-performance anion-exchange with PAD (Dionex, Sunnyvale CA). The high-performance anion-exchange with pulsed amperometric detection instrument consisted of an ED40 electrochemical detector, a GP40 gradient pump, an AS50 autosampler, and a Carbopac PA-10 guard and analytical column (all from Dionex). Glucose, xylose, arabinose, galactose, and mannose were separated on the analytical column using ultrapure water as mobile phase, at a flow rate of 1.0 mL/min. In order to enable detection of the sugars using PAD, 600 m*M* NaOH was added by a postcolumn pump.

Determination of Acetyl Groups

The analysis of acetyl groups was based on the release of acetyl groups by alkaline treatment *(24)*. Fractions collected during SEC were first freeze-dried and then dissolved in 1 mL NaOH (1%) solution to remove acetyl

groups from the polysaccharide by 12-h incubation at room temperature. The concentration of liberated acetyl groups was determined by HPLC as acetic acid. Bound acetyl content was calculated by subtracting the free acetate amount in the sample from the amount quantified after treatment with 1% NaOH.

Acetic acid was determined using a GE Healthcare HPLC system equipped with an RI detector. An Aminex HPX-87H organic acid column (BIO-RAD, Hercules, CA) thermostated at 65°C was used to separate acetic acid from other compounds. The mobile phase was 5 mM H_2SO_4 at a flow rate of 0.6 mL/min. The system was equipped with a Cation-H Refill Cartridge (BIO-RAD) to protect the analytical column.

Calculations

Yield of Xylan in Heat-Treated Samples

Polymer form of extracted hemicellulose was expressed on the basis of polymeric xylose, i.e., xylan. The xylan recovery, i.e., yield of hemicellulose extracted from SFCF, was calculated according to Eq. 1.

$$Y_{\text{xylan}} = \frac{X_{\text{H}} - X_{\text{F}}}{f \times X_{\text{T}}} \times 100 \tag{1}$$

where, X_{H} is the xylose content, calculated in grams, determined after acid hydrolysis of the filtrate obtained in microwave treatment, X_{F} is the xylose content in the filtrate calculated in grams based on direct sugar analysis of the filtrate obtained in treatment, f is the conversion factor of polymer to monomer hydrolysis (for xylose-based polymers f = 1.136 g/g), and X_{T} is the amount of xylan in grams in SFCF used in the experiments.

Weight-Average Molecular Weight

A generally accepted formula for the calculation of weight-average molecular weight of polymers *(25)*, based on data obtained from SEC chromatograms is shown in Eq. 2.

$$M_{\text{W}} = \frac{\sum_{i=1}^{N} \left(c_{\text{i}} \cdot M_{\text{i}}\right)}{\sum_{i=1}^{N} c_{\text{i}}} \tag{2}$$

where M_{i} is the molecular weight at elution volume v_{i} and c_{i} is the concentration at elution volume v_{i}. The concentration c_{i}, was calculated from the acid hydrolysis of fractions collected during SEC, whereas M_{i} was calculated from the SEC dextran calibration according to Eq. 3 *(16)*.

$$\log M_{\text{i}} = 0.0968 \times v_{\text{i}} + 6.7103 \tag{3}$$

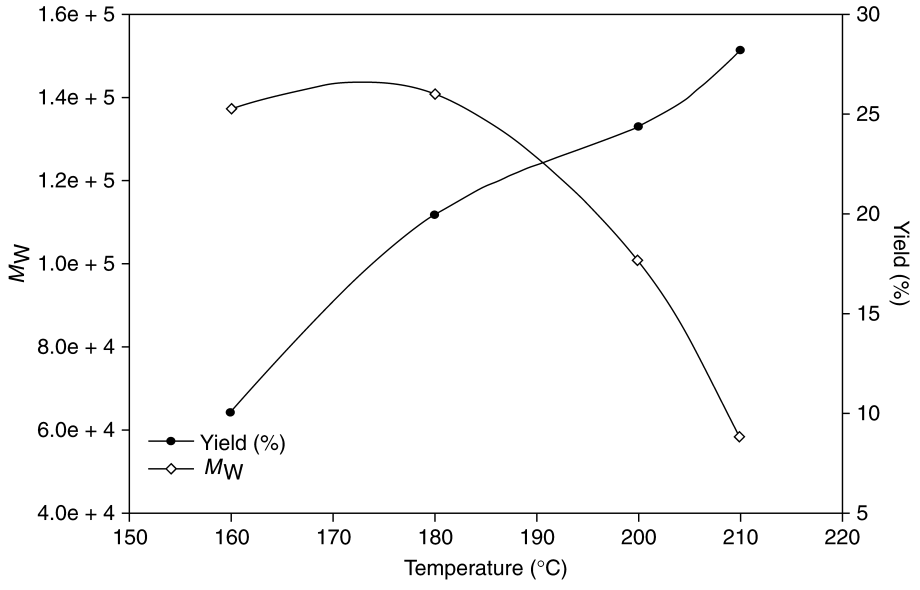

Fig. 2. Effect of temperature on the molecular weight and yield of hemicellulose extracted by microwave assisted heat treatment using pure water.

Results and Discussion

Effect of Temperature

The effect of temperature on the isolation of hemicelluloses from SFCF was examined applying water as the extraction agent without any addition of acidic or alkaline catalysts. Treatment temperature was varied between 100°C and 210°C, which was the highest working temperature of the microwave oven. Water-soluble hemicellulose fractions obtained at different temperature set points were analyzed for molecular weight with SEC. Polymeric hemicellulose recovery was calculated on the basis of xylan recovered (Fig. 2). Treatments carried out at 100°C and 130°C did not produce measurable amounts of solubilized hemicellulose. About 10% hemicellulose extraction, with a polymer M_w of 1.4×10^5, was reached at 160°C. Increasing treatment temperature significantly influenced the hemicellulose recovery. A maximum of 22.8% hemicellulose yield was obtained at the highest (210°C) temperature value. However, severe degradation of the isolated hemicellulose was observed at higher treatment temperatures, resulting in the lowest M_w of about 6×10^4 at 210°C. The white–offwhite color of hemicellulose isolates obtained at 160°C and 180°C turned to brown at elevated treatment temperatures.

Optimization of the microwave-assisted heat treatment has to be a compromise between hemicellulose recovery and the molecular weight of the isolated fraction. As shown in Fig. 2, applying higher temperatures than 180°C will significantly lower the molecular weight of isolated

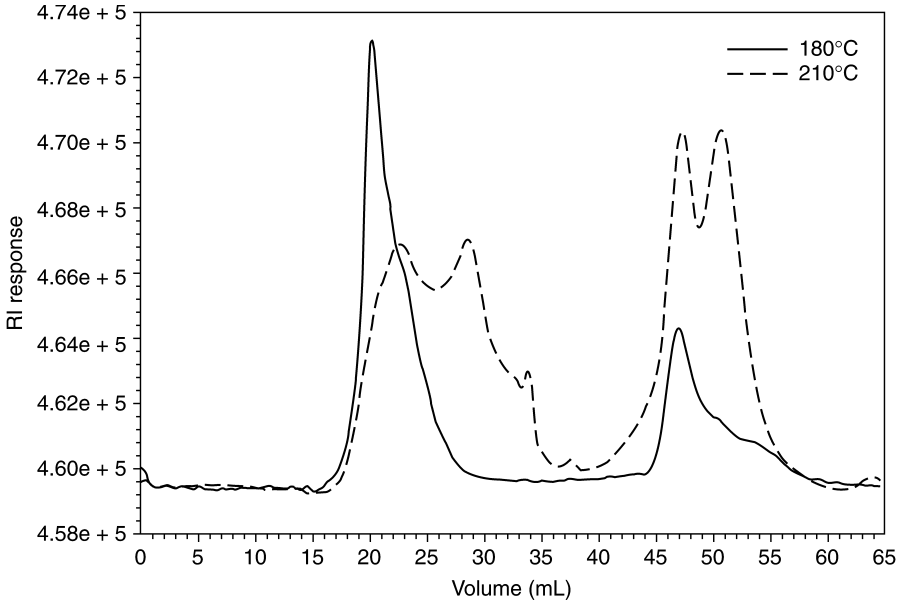

Fig. 3. SEC RI chromatograms of hemicellulose samples extracted by microwave assisted heat treatment at 180°C and 210°C using pure water.

hemicellulose. Furthermore, whereas the hemicellulose yield was doubled by a temperature increase from 160°C to 180°C, with no apparent degradation of polymer chains, only 20% higher yield was obtained by another 20°C temperature increase. The pH of the suspension after heat treatment was measured. Compared with the initial pH of 6.2, significantly lower pH values were observed. Increasing treatment temperature resulted in lower pH, for example, at 160°C pH of 5.6, at 180°C pH of 4.3, and at 210°C pH of 3.7 was measured.

The presence of acetyl groups on many types of hemicelluloses present a specific problem. During heat-treatment at certain conditions the acetyl groups can be released yielding acetic acid, thereby causing a decrease in pH. The lower pH, in turn, may cause acid hydrolysis of the glycosidic bonds in the polysaccharide backbone and/or substitutions. In the current case, the pH of the mixture measured after treatment indicated that liberation of acetic acid occurred to a greater extent at high treatment temperatures. These data suggest that acid hydrolysis became the dominant mechanism of hemicellulose recovery over physical extraction of hemicellulose.

Two SEC chromatograms of polysaccharides isolated at 180°C and 210°C are shown in Fig. 3. It can be seen that at 180°C, two dominant fractions were found in the extracts. The peak area of the first peak resulting from the elution of high-molecular weight polymers is significantly greater than the area of the second peak, indicating that the extract contained higher amounts of high-molecular weight polysaccharides. On

Table 1
Molecular Weight and Yield of Microwave Isolated Hemicellulose (at 180°C, Impregnated With Water, Acid, or Alkali) and Alkaline Extracted, Ethanol Precipitated Hemicellulose-B

Treatment Type	Concentration of impregnation media (%)	Molecular weight (M_w)	Yield (%)
Water	–	127,000	20
H_2SO_4	0.025	13,800	3.9
H_2SO_4	0.5	17,600	2.2
NaOH	0.025	172,000	4.6
NaOH	0.5	136,000	11
Alkaline extracted ethanol precipitated hemicellulose-B	–	192,000	56

the other hand, the extract obtained at 210°C showed more diversity and at least four fractions could be identified. Lower molecular weight polysaccharides were more prominent in this extract.

Effect of pH

Heat treatment of SFCF applying acid or alkaline catalyst was performed at 180°C. Before heat treatment the material was impregnated as written in Materials and Methods section. Both hemicellulose recovery and molecular weight of the isolated hemicellulose were determined (Table 1). The addition of acid did not improve hemicellulose recovery, and considerably lower hemicellulose yields were obtained relative to impregnation with pure water. Not surprisingly, the molecular weight of the recovered hemicellulose fraction was also lower in the presence of the acid catalyst. Furthermore, increasing the acid concentration did not affect hemicellulose molecular weight or recovery. Sodium hydroxide seemed to be a more suitable impregnation agent than sulfuric acid. The molecular weight of the hemicellulose isolates was greater than in case of water extraction; however, hemicellulose yields were far less than those achieved with pure water (Table 1).

Comparison of Microwave and Alkaline-Extracted Hemicellulose

Hemicellulose polymers fractionated by microwave-assisted heat treatment of SFCF were compared with alkaline-extracted and ethanol-precipitated hemicellulose-B. Heat treatment was carried out at 180°C with 5 min of holding time using pure water, whereas hemicellulose-B was isolated as described in the Materials and Methods section of this paper. Molecular weight, acetyl group content, and recoveries based on the

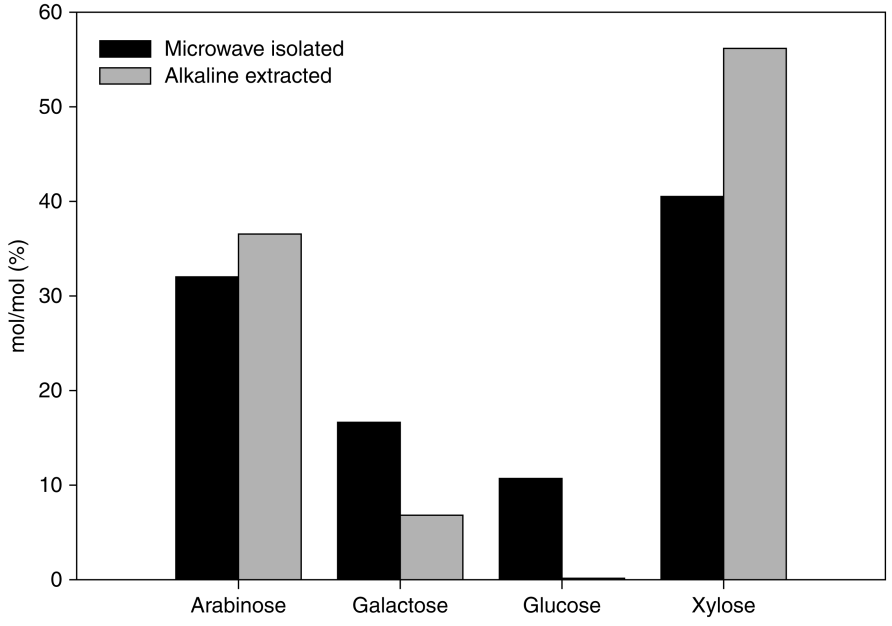

Fig. 4. Sugar composition of hemicellulose isolated in microwave assisted heat treatment at 180°C with pure water and alkaline extracted and precipitated hemicellulose-B. (The amounts of different sugars are expressed in the percent of the total sugar content of the sample in moles.)

original raw material xylan content were examined. The molecular weight of hemicellulose-B was higher than that achieved on microwave heat treatment (Table 1). This could be explained with the lower applied temperature of the alkaline treatment; second, liberated acetic acid was neutralized by the sodium hydroxide.

The analysis of fractions during SEC revealed some differences in the composition of polysaccharides obtained in the two preparations. The two dominating sugar residues were xylose and arabinose in both cases (Fig. 4) indicating that, as expected, arabinoxylan is the major polysaccharide. The molecular ratios of xylose : arabinose were also similar: 1.5 for the alkaline extract, and 1.3 for the microwave extract. These values are close to the reported ratio of approx 1.4 for corn hemicellulose *(12)*. The galactose content in the alkaline preparation is also very close to that previously reported for corn fiber hemicellulose (approx galactose : xylose ratio of 0.1). However, the microwave extracted preparation contained a significantly higher amount of galactose (galactose : xylose ratio 0.4). Furthermore, the microwave preparations contained glucose (Fig. 4), possibly indicating minor contamination of starch.

The SEC chromatograms of the two hemicellulose isolates are shown in Fig. 5. Two distinctly separated peaks were observed for both hemicellulose isolates. The analysis of fractions collected during the elution of the first peak, representing high-molecular weight polysaccharides,

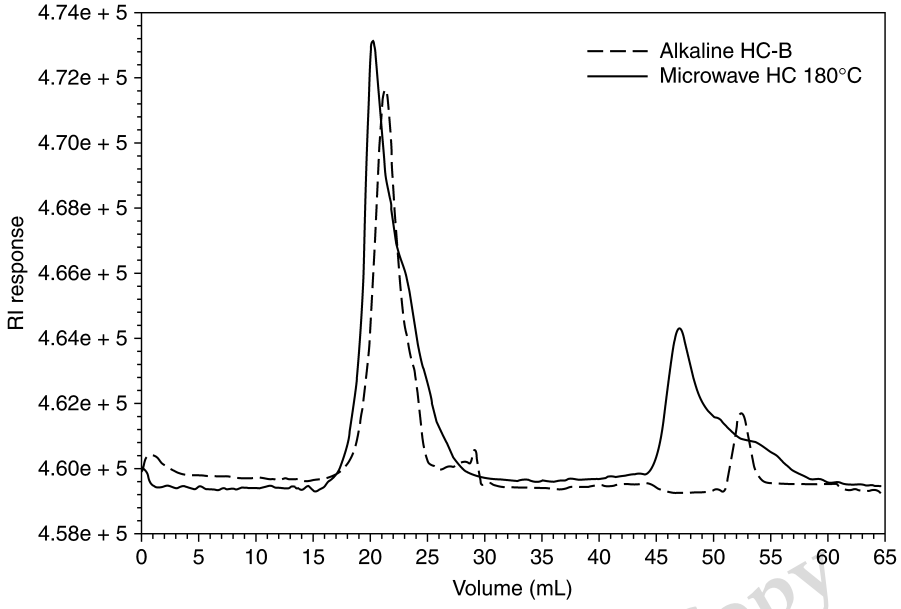

Fig. 5. SEC RI chromatograms of hemicellulose extracted by microwave assisted heat treatment at 180°C with pure water and hemicellulose-B.

showed that these polysaccharides were mainly made up of xylose and arabinose, their ratio was 2.2 for the microwave extracted preparation and 2.0 for alkaline treatment. On the other hand, fractions collected later, i.e., the smaller molecular weight polysaccharides, were rich in arabinose. The xylose content of these fractions was about half of the arabinose measured.

Alkaline-extracted hemicellulose-B did not contain a measurable amount of acetyl groups. However, the acetyl group content of hemicellulose isolated on heat treatment at 180°C using around 3 wt% water for the extraction. It is noteworthy to mention that acetyl groups were only detected in those fractions eluted during SEC, which corresponded to the peak containing higher molecular weight polymers. Further experiments will be performed to analyze the assumed position of acetylation.

Conclusion

The aim of this study was to investigate the possibility of hemicellulose-based polysaccharide isolation from SFCF in microwave-assisted heat treatment and to compare the isolated polysaccharide with hemicellulose-B, a polymer obtained with the well-known alkaline extraction-ethanol precipitation method. The results of aqueous hemicellulose extraction performed at different temperatures showed that hemicellulose recovery could be improved by increasing the temperature of the treatment, but at the same time the molecular weight of the isolated polymers, along with the pH of

the reaction mixture decrease, supporting the assumed mechanism of degradation of polymer chains caused by autohydrolysis.

Application of acid catalyst (sulfuric acid) did not increase hemicellulose recovery and very low molecular weights were obtained. Sodium hydroxide proved to be a more sufficient catalyst. Although, hemicellulose yields were less than those obtained with water, higher molecular weight polysaccharides were isolated. In terms of comparison with alkaline extraction, it could be concluded that microwave treatment provided lower molecular weight and lower polymer recovery, but on the other hand it performed well without application of any chemicals. Thus, it could be a novel environmentally sound way of hemicellulose isolation.

Acknowledgments

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