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Fractionation of wheat straw meal after pretreatment with acidified zinc chloride solutions

Ivan Šimkovic, Miroslav Antal & Juraj Alföldi

Institute of Chemistry, Slovak Academy of Sciences, 84238 Bratislava, Slovak Republic

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Wheat straw was treated with acidified $ZnCl_2$ solutions and the fractions obtained were compared with results obtained by NaOH treatments. The yields of water-soluble polysaccharides are smaller but they are less contaminated with lignin than analogous fractions obtained by NaOH treatment. The molecular weights of the $ZnCl_2$ fractions are greater than the NaOH-treated fractions. The arabinose and glucuronic acid residues are hydrolysed from the polysaccharides by $ZnCl_2$ treatment. Samples treated with $ZnCl_2$ could be more effectively desalted by washing with ethanol than by precipitation in acetone. NaOH-pretreated samples could not be desalted by washing with ethanol.

INTRODUCTION

Wheat straw represents an important agricultural material which is annually produced and consumed. It contains 21 lignin, 35% cellulose, 25% xylan and 1% mannan (Baehr *et al.*, 1991). This material could be used for D-xylose (Whistler, 1950), furfural (Carrasco, 1991), energy (Mirau, 1991) and feed (Hemling *et al.*, 1990; Swanson *et al.*, 1990) production. It is known that wheat straw xylan contains from 3 to 7·9% uronic acid residues and up to 6·9% arabinose (Bishop, 1953; Aspinall & Mahomed, 1954). In relation to our previous work on fractionation of lignocellulose materials (Šimkovic *et al.*, 1992) we decided to also explore this source.

During the last few years some activities could be observed in the modification of lignocellulose materials and cellulose by pretreatment with zinc chloride (Chen, 1991; Grinshpan *et al.*, 1991; Kirubaharan *et al.*, 1991; Saitô *et al.*, 1991). Aqueous solution of this salt was used for the swelling of cellulose (Inagaki *et al.*, 1976) prior to chemical modification and could be eluted from the material by washing with 96% ethanol. It indicates that in this way the polysaccharide fractions could be desalted and separated from lignin during the fractionation of plant material. Drastic degradation of cellulose could be caused by treatment with zinc chloride in aqueous phenol (Ferrier *et al.*, 1992). This Lewis acid is also a known fire-retarding additive which suppresses the flaming combustion by reducing the rate of production and total amount of combustible volatiles (Shafizadeh *et al.*, 1978). It seems that zinc chloride acts as a catalyst for the condensation reactions of the volatiles formed.

In the present study we wanted to find out how effective zinc chloride pretreatment could be for the fractionation of hemicelluloses from wheat straw in comparison to previously used methods (Šimkovic *et al.*, 1990, 1991).

EXERIMENTAL

The wheat straw was milled to particles smaller than 0·7 mm prior to the treatment. It contained 21·7 Klason lignin, 3·68% ash and 0·9% water. We used seven fractionation procedures which should cover the aspects of the zinc chloride effect on the properties of the fractions obtained.

According to the first fractionation procedure (P1) material was activated with 50% zinc chloride solution prepared by acidification to pH 1 with crystalline orthophosphoric acid. The treatment started with an initial 1:10 w/v wheat straw/solution ratio and lasted 50 h at

40°C under reduced pressure (about 20 Pa). During that time water was added several times when the material started to become viscous. The water soluble portion was filtered, dialysed through tubing with 8000–15 000 Da exclusion limit and freeze dried.

The second procedure (P2) was based on mixing of 10 g of wheat straw meal with a solution consisting of 120 g of $ZnCl_2$, 68 ml of water and 5·1 ml of concentrated HCl. The mixture was treated for 2 h under reflux (130°C), diluted with 96% ethanol, filtered and washed with ethanol till there was a negative reaction on chloride ($AgNO_3$). Then the residue was washed with water and divided into the water-soluble part and the insoluble residue.

According to the P3 procedure 10 g of straw was treated with 2 litres of 5% acidified $ZnCl_2$ solution under reduced pressure and after evaporation of water the material was washed with 80% ethanol and the residue with hot water. The eluants obtained were dialysed and lyophylized.

Procedure P4 (Antal *et al.*, 1984) consisted of pretreatment with 17·5 NaOH solution and subsequent extraction with 80% ethanol, water and 5% NaOH.

In the fifth procedure (P5; Šimkovic *et al.*, 1992) we used 5% NaOH containing 1% H_2O_2 for extraction.

When 5% zinc chloride solution containing 5% H_2O_2 adjusted to pH 3·85% was utilized for fractionation (P6), wheat straw (10 g) was mixed with 200 ml of prepared solution and vacuum-evaporated. The residue was mixed and washed with acetone till there was a negative reaction on chloride ($AgNO_3$). Then the residue was divided into the water-soluble and insoluble parts by washing with water.

The last procedure (P7) consisted of vacuum-evaporation treatment of wheat straw (10 g) with 200 ml of 5% $ZnCl_2$ acidified with HCl. The residue obtained was diluted with water and the water soluble part precipitated into acetone. The precipitate was dissolved in water and precipitated again two more times. Subsequently the precipitate was diluted in water and freeze dried.

The molecular weights of water-soluble samples were determined osmometrically (Knauer, steam method) with the help of dextran standards. All other methods have been described previously (Šimkovic *et al.*, 1992).

RESULTS AND DISCUSSION

Table 1 lists the results of fractionation procedures. The P1 fractionation with 50% $ZnCl_2$ gives a water-soluble portion with high Klason lignin and ash contents. With this procedure zinc chloride could not be dialysed from the eluant, most probably because of its conversion to insoluble zinc oxide. This sample had $\overline{M}_n = 23\,628$ and the ^{13}C-NMR spectrum in DMSO-d_6 showed only five signals for xylopyranose units (102·0, C-1; 72·9, C-2; 74·2, C-3; 75·9, C-4; 63·4 ppm, C-5). The same sample when analysed in D_2O showed only some xylose-containing oligomers (103·0, C-1 of non-reducing xylose units; 97·7 and 93·3 ppm, C-1 of β- and α-anomers of reducing xylose units; Bock *et al.*, 1983). This indicates that although the fraction could be isolated from the material in this way, after dialysis of the acid it was only partially soluble in water.

Table 1. Yields, Klason lignin and ash contents obtained by fractionation procedures from wheat straw

Procedure[a]/ fraction	Yield (%)	Klason lignin (%)	Ash content (%)
P1/H_2O	11·4	15·4	34·8
P1/Residue	89·6	21·1	3·4
P2/H_2O	0·9	—	—
P2/Residue	63·3	47·5	5·3
P3/80% C_2H_5OH	5·4	11·6	16·3
P3/H_2O	9·9	3·6	2·6
P3/Residue	66·0	32·0	4·2
P4/80% C_2H_5OH	8·7	74·8	4·0
P4/H_2O	32·5	7·0	19·5
P5/5% NaOH	9·8	1·2	28·3
P4/Residue	39·4	12·1	21·9
P5/H_2O	27·7	23·9	7·3
P5/Residue	50·6	21·1	3·1
P6/H_2O	3·3	7·5	19·0
P6/Residue	85·2	17·4	3·5
P7/H_2O	9·1	3·9	19·5
P7/Residue	81·8	25·1	3·0

[a]For details see Experimenal section.

When wheat straw was treated according to the P2 procedure then the yield of water-eluant obtained after elution with ethanol was less than 1% from starting material (Table 1). According to the ^{13}C-NMR spectrum it contained only xylo-oligosaccharides. The residue obtained gave a Klason lignin value which we believe was higher than the real value due to carbonization (carbon content was 54·3%) during the refluxing procedure. These conditions were used for solubilization of cellulose (Chen, 1991) but are too drastic for fractionation.

When 5% $ZnCl_2$ was used for the fractionation (P3, Table 1) then the total yield obtained by elution with ethanol and water was higher than materials eluted with water in the P1 procedure (treatment with 50% $ZnCl_2$). The ethanolic eluant (11·6% of Klason lignin) also contained some xylose-containing fragments with ^{13}C-NMR anomeric signals at 103·6, 102·2, 100·0, 97·9 and 92·7 ppm (Bock *et al.*, 1983). The ash content of this fraction was 16·3%, which indicates the presence of zinc compounds. The fraction obtained subsequently by elution with water had $\overline{M}_n = 18\,835$ and contained, according to ^{13}C-NMR data, besides xylan (102·8, C-1 of unsubstituted xylose; 102·5, C-1 of xylose substituted with D-glucuronic acid and 98·9 ppm, C-1 of D-glucucronic acid unit) also some glucomannan (103·7, C-1 of

glucose unit and 100·9 ppm, C-1 of mannose unit; Bock *et al.*, 1983) which was not present when 50% ZnCl was used. The Klason lignin and ash contents of this fraction were small. The residue after fractionation contained much more Klason lignin than the isolated fractions and had ash contents close to the value of the starting material.

When the material was pretreated with 17·5% NaOH, the procedure used previously on corn cobs, bagasse or Black Poplar (Šimkovic *et al.*, 1990, 1991, 1992), then the yields obtained with wheat straw were greater (Table 1, P4). The ethanolic eluant had a high Klason lignin content which could also be confirmed by the ^{13}C-NMR spectrum measured in DMSO-d_6. It contained an anomeric carbohydrate signal (102·8 ppm) and the lignin signals (153·1, C-4G-CHO, esterified; 148·2, C-3 guaiacyl; 134·7; 130·1, C-1 of guaiacyl; 122·7, C-6G-CH_2; 116·5, C5 of β-arylethers; 112·1, C-2G-CH=CH-; 105·1, syringyl substituted at positions 2 and 6; 56·8, OCH_3; and 30·9 ppm, aliphatic carbon not bonded to oxygen; Nimz *et al.*, 1982; Kringstad & Mörk, 1983). The water soluble fraction contained 7·0% of Klason lignin and the yield was 32·5% with an ash content of 19·5%. It seems that the sodium salts introduced by NaOH treatment could not be eluted by ethanol like zinc compounds. The lower molecular weight ($\overline{M}_n = 9151$) obtained in this way indicates more severe degradation than on fractions obtained by the previous methods. The ^{13}C-NMR spectrum contains two anomeric signals when measured in D_2O, 102·7 and 108·8 ppm. The first one belongs to unsubstituted xylose and the second one to arabinose units (Kováč *et al.*, 1982; Schraml *et al.*, 1984). The residue obtained is the smallest from all procedures used but close to previous results obtained on corn cobs and bagasse (Šimkovic *et al.*, 1990, 1992). When a similar procedure was run on Black Poplar then about 60% of the starting material remained (Šimkovic *et al.*, 1991).

The treatment of wheat straw with 5% NaOH containing 1% H_2O_2 (Table 1, P5) gave similar results to those obtained on corn cobs (Šimkovic *et al.*, 1992). Although the yield obtained by this method is big, its Klason lignin content is the greatest from all water-soluble fractions obtained. Its $\overline{M}_n$ value is 14 300 and its ^{13}C-NMR spectrum (in D_2O) was very similar to the one obtained on the water-soluble fraction from procedure P4. This method is not suitable for the separation of lignin from hemicelluloses.

When zinc chloride was treated with hydrogen peroxide under acidic conditions (Table 1, P6), then the yield of water-soluble fraction was the lowest from all procedures used previously. According to the Klason lignin content this fractionation degraded more lignin than H_2O_2 without $ZnCl_2$ under alkaline conditions. The ash content indicates that the water-soluble fraction was not separated from zinc compounds. The results were about the same when 96% ethanol was used instead of acetone. The ^{13}C-NMR spectrum confirmed the presence of arabinoglucuronoxylan as well as glucomannan. This composition is similar to the water-soluble fraction obtained by treatment with 5% $ZnCl_2$ (procedure P3).

Treatment with 5% $ZnCl_2$ combined with precipitation in acetone (Table 1, P7) gave about the same yield of water-soluble fraction as obtained with the P3 extraction. More material remained in the residue due to the vacuum evaporation treatment. The precipitation procedure was less effective in isolating salts from polysaccharide than washing with ethanol prior to water. The osmometrically determined molecular weight of P7/H_2O ($\overline{M}_n = 10214$) was lower than that observed for the P3 treatment while the NMR data were similar. It seems that the difference in zinc chloride and lignin contents affected the resulting molecular weights.

CONCLUSIONS

The fractionation of wheat straw after zinc chloride pretreatment gives smaller yields of polysaccharide fractions than the NaOH methods used previously. The procedure with 5% $ZnCl_2$ gave the smallest lignin content from all water-soluble fractions. The molecular weights determined on $ZnCl_2$-treated fractions were bigger than those obtained by NaOH-fractionation procedures. The composition of the water-soluble fractions differ mostly in lignin, glucomannan, arabinose and glucuronic acid contents as analysed by ^{13}C-NMR spectroscopy. The best method for separation of $ZnCl_2$ from polysaccharides is washing with ethanol while 5% $ZnCl_2$ concentration is sufficient for the treatment as far as the yield of fractions obtained.

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