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Isolation, Extraction and Purification of Hemicelluloses of *Ceiba pentandra* and *Morus nigra*

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ABSTRACT: The hemicelluloses are the principle non-cellulosic polysaccharides present in wood. Hemicellulose and cellulose collectively make the holocellulose of the wood. Hemicelluloses are distinguished from cellulose by the property that they are soluble in dilute aqueous alkali and in boiling water. After separation from the other wood components these are hydrolysed by warm and dilute acids into simple pentose or hexose sugars. Xylan, araban, mannan and galactan, as the name imply; on hydrolysis each one gives xylose, arabinose, mannose and galactose respectively. A hemicellulose giving both galactose and arabinose is a galacto-araban. The hemicelluloses are completely soluble in dilute caustic alkalis. A cold or hot 5% solution of sodium hydroxide is usually employed for their extraction. Isolated hemicelluloses are also soluble in hot, saturated solution of sodium carbonate and pentosans are removed from wood pulp by heating it with magnesium sulphite in the presence of calcium or magnesium oxide.

Keywords: Hemicellulose, holocellulose, hexose, pentose and wood pulp.

INTRODUCTION

The term hemicellulose was introduced by Schulze [23]. He defined hemicelluloses as those carbohydrates occurring in the plant cells that are dissolved by dilute alkalis and are readily hydrolyzed by hot dilute mineral acids. The hemicelluloses are anhydrides of hexose and pentose sugars. Xylan, araban, mannan and galactan, as the name imply; on hydrolysis each one gives xylose, arabinose, mannose and galactose respectively. A hemicellulose giving both galactose and arabinose is a galacto-araban. Pelouze [20] pointed out that contrary to the general opinion, a boiling dilute mineral acid acting on paper, linen and saw dust produces some glucose. The hemicelluloses are completely soluble in dilute caustic alkalis. A cold or hot 5% solution of sodium hydroxide is usually employed for their extraction. Isolated hemicelluloses are also soluble in hot, saturated solution of sodium carbonate [15 & 18]. Schwalbe [24] removed the pentosans from wood pulp by heating it with magnesium sulphite in the presence of calcium or magnesium oxide.

The hemicelluloses have a much lower DP than cellulose, being about 155, compared to a maximum 400 or higher for native wood cellulose [16]. The molecular weights of hemicellulose have been reported [8] to be of the range of 20,000. Hemicelluloses are more heterogeneous than cellulose. Unlike cellulose, which yields only glucose on hydrolysis, hemicellulose hydrolyzes to a variety of saccharide units. One distinction sometimes made is to divide the hemicelluloses into two broad classes - the cellulosans and the polyuronides; the cellulosans include all those hemicelluloses which are built up of simple sugars and which include both the hexosans and the pentosans. The hexosans are represented by mannan, galactan and glucosan where as the pentosans, as xylan and araban. In hardwoods, the dominant hemicellulose is xylan, which is made up of the pentose of five carbon sugars and xylose [19]. Xylan is also present in softwood hemicellulose, but there is also an appreciable quantity of the hexosans and mannan present in softwoods. The cellulosans are strongly associated with the cellulose in wood and are not easily removed by alkali. Mannan tends to associate very closely with cellulose. These accounts for the difficulties in extracting all mannan form the alpha-cellulose of certain softwoods. Polyuronides are hemicelluloses which contain large amount of hexuronic acids along with some methoxyl and acetyl groups and some of

the free carboxylic acid groups. The polyuronides are mainly associated with lignin, although some association of it exists with the cellulose [2]. Two main types of polyuronides are now recognized: 4-*O*-methyl-glucurano-xylan, in the case of hardwoods, and 4-*O*-methyl-glucurano-arboxylan in case of softwoods.

Hemicelluloses remaining in the pulp play an important role in paper making, particularly with regard to fibre preparation and fibre bonding [14 & 27]. Hemicelluloses, particularly those containing hexuronic acids grouping, impart hydrophilic properties to pulp fibres which increase the rate of beating of pulp and also improve the tensile and bursting strength of the paper. On the other hand hemicelluloses are believed to be partly responsible for the loss in brightness of pulp on aging. Hemicelluloses are undesirable in chemical pulps for rayon, cellophane, cellulose acetate or cellulose ether, where they result in 'Haze' formation and filtration difficulties. They must be removed as completely as possible from these pulps. The non-cellulosic polysaccharides (hemicelluloses) are the main constituents of wood, while studying the chemical composition of non-cellulosic polysaccharides, the main objectives of a chemist is to isolate, fractionate and purify the polymers to a reasonable extent with minimum possible degradation. It has been observed that the fractionalization and purification of the extracted hemicelluloses are sometime very difficult. In view of achieving the above mentioned objectives more efficient and advanced methods of isolation and fractionalization of the non-cellulosic polysaccharides are required.

MATERIAL AND METHODS

Ceiba pentandra and *Morus nigra* wood dust samples were taken separately for various steps of the analysis:

2.1 Preparation of extractive free wood dust: Approximately 1.0 kg of 40 to 60 mesh wood dust was extracted with benzene and alcohol mixture in the ratio of 1:2 for several hours to attain complete removal of alcohol-benzene extract. Then the extracted wood dust was air dried and followed by extraction with about 5.0 liters of hot water at 70-80^0 C for about five hours. The content obtained was filtered in muslin cloth and it was dried in air.

2.2 Preparation of holocellulose from extractive free wood dust: An approximate quantity of 100.0 gm of extractive free, dried dust was taken in a five liter capacity conical flask. Two liters of distilled water was taken in other conical flask, and 24.0 gm of sodium chloride, 50.0 ml of glacial acetic acid were added to it and dissolved. Then this solution poured into the first flask containing the extract free dried wood dust. The contents of the conical flask were heated for about one hour at 70±5^0 C, then again 40.0 gm of sodium chloride and 50.0 ml of glacial acetic acid were added into five liter conical flask and heating process was repeated 4 to 5 times to attain the desired lignin removal. The holocellulose was obtained from *Ceiba pentandra* and *Morus nigra* wood dust, both these wood contain lignin upto 2.62 and 2.08 respectively. Lastly the content of the conical flask was filtered and the residue was washed thoroughly with distilled water. The final washing was done with acetone and the residue was air dried. The above process was repeated several times (nearly ten times). About half a kilogram of hemicellulose was finally obtained and it was chemically analyzed according to various methods as Official Standard [19] given by TAPPI, Wise [29] and Dore [7].

2.3 Preparation of hemicelluloses from holocellulose[3]: Approximately 500.0 gm moisture free holocellulose of *Ceiba pentandra* and *Morus nigra* was extracted in batches separately.

2.3.1 First extraction with 5% alkali: About 100.0 gm of dried, moisture free holocellulose was taken in a four liter conical flask, filtered with a funnel having an inlet and outlet openings to create inert atmosphere during reaction and also provided with a stirrer. The air present in the flask was replaced by passing the nitrogen gas through the flask for about twenty minutes. At 20^0 C, about three liter of 5% oxygen free KOH solution was added through groping funnel into the conical flask and the temperature was maintained at 20^0 C by stirring the material. The flask was kept for two hours and content was stirred repeatedly for completion of the reaction. Then the reaction mixture was filtered through a fine cloth kept in Buchner funnel and residue was washed first with 5% oxygen free KOH solution and then with

distilled water. The filtrate thus obtained through two washing was mixed together. The residue was served for further extraction.

2.3.1.1 Precipitation of hemicellulose in the filtrate and washing solution: First of all the pH of the filtrate and washing solution was maintained below 5.5 by adding sufficient amount of glacial acetic acid and during this treatment slight turbidity was observed. Absolute alcohol was added in the solution, till hemicellulose was completely precipitated. For checking of complete precipitation, little amount of absolute alcohol was added and no appreciable precipitation was taken place. Then the precipitate was allowed to settle down by standing the content of conical flask overnight. The supernatant liquid was removed by siphon and the precipitate of hemicellulose was filtered and washed with 50, 70 and 90% concentrations of ethanol and finally with absolute alcohol. Following the above treatment the precipitate hemicellulose were freeze dried in Lyophilizer [28]. (Extreme precautions were taken by avoiding exposure of sample to atmosphere before complete drying, because moisture causes the hornification of hemicellulose sample).

2.3.2 Second extraction with 16% alkali: For further extraction, the residue obtained after first extraction was air dried. Then the residue was treated with 16% KOH solution free from oxygen, and same processes and conditions of first extraction was repeated. The residue of second extraction was preserved for further extraction. Glacial acetic acid was added in the filtrate for acidification and the pH was maintained below 5.5, using excess of ethanol. The precipitate of hemicellulose was left overnight to settle down. The precipitate or hemicellulose content was separated from the solution by the procedure explained for first extraction. The moisture was removed using sequence treatment of alcohol and finally washed with other. Lastly, the precipitate sample was dried under vacuum using freeze drier.

2.3.3 Third extraction with 24% alkali and 4% boric acid: The residue obtained after second extraction was treated with two liter of oxygen free 24% KOH solution plus 4% boric acid under similar condition described during the first extraction. The same procedure of first extraction was followed here also for filtration, precipitation, washing and drying. The precipitate fraction of hemicellulose obtained was relatively low in amount in third extraction. The residue of third extraction was mainly cellulose content. The hemicellulose fraction were dissolved in 10% KOH solution in about 1.25 concentration and then measured the optical rotation of hemicellulose fractions using modern Perkin-Elmer-Spectro polarimeter[11]. This instrument is fitted with a holographic grating monochromator and has double beam operation. It is controlled by microcomputer, which allows the analyst to carry out repetitive scans of spectrum, measure absorbance at specified wave lengths and read concentration directly.

2.4 Purification of non-cellulosic polysaccharides i.e. hemicellulose fractions: For purification [4] of the various fractions of hemicelluloses, improved process of Chanda et al.[5] was followed. About 60.0 gm of 5% KOH hemicellulose fraction was dissolved in 10% of one liter of oxygen free alkali solution. Followed by this, the solution was neutralized with glacial acetic acid and the pH was maintained below 5.5. The precipitation was carried out by adding excess of ethanol. The precipitate was then filtered, washed and redissolved in alkali. The process was repeated twice. The hemicellulose fraction was again dissolved in 10% alkali solution and freshly prepared Fehling solution was added with constant stirring. A small quantity of acetone, about 10.0 ml, added for fast setting of precipitate (hemicellulose content). Then the precipitate was filtered and washed with distilled water. The precipitate thus obtained was dispersed in about two liters of distilled water with constant stirring. In this process copper complex was produced. It is decomposed by adding little amount of 2N HCl and solution was poured into absolute alcohol of about double of its volume. The white precipitate produced was filtered and washed with 1N HCl in a mixture of acetone and water (6:4) in order to remove copper completely. The precipitate of hemicellulose was further washed with mixture of alcohol and water. The water was removed from the precipitate by using different concentrations of alcohol in increasing order and then with absolute alcohol. Finally the precipitate was washed with ether and completely dried in freeze-drier. By this process purified hemicellulose was obtained.

RESULTS AND DISCUSSION

The observed data of chemical analysis of holocellulose of *Ceiba pentandra* and *Morus nigra* is recorded in Table 1 and compared through the Figure 1.

Table 1: Chemical analysis of holocellulose.

Sr. No.	*Ceiba Pentandra* (%)	*Morus Nigra* (%)
Ash content	2.98	2.56
Lignin content	2.62	2.08
Acetyl value	1.54	2.24
Methoxyl value	2.30	4.72
Pentosan content	19.58	15.02
Alpha-cellulose content	42.00	44.08
Hemicellulose content	22.23	23.68
Holocellulose content	60.78	69.00

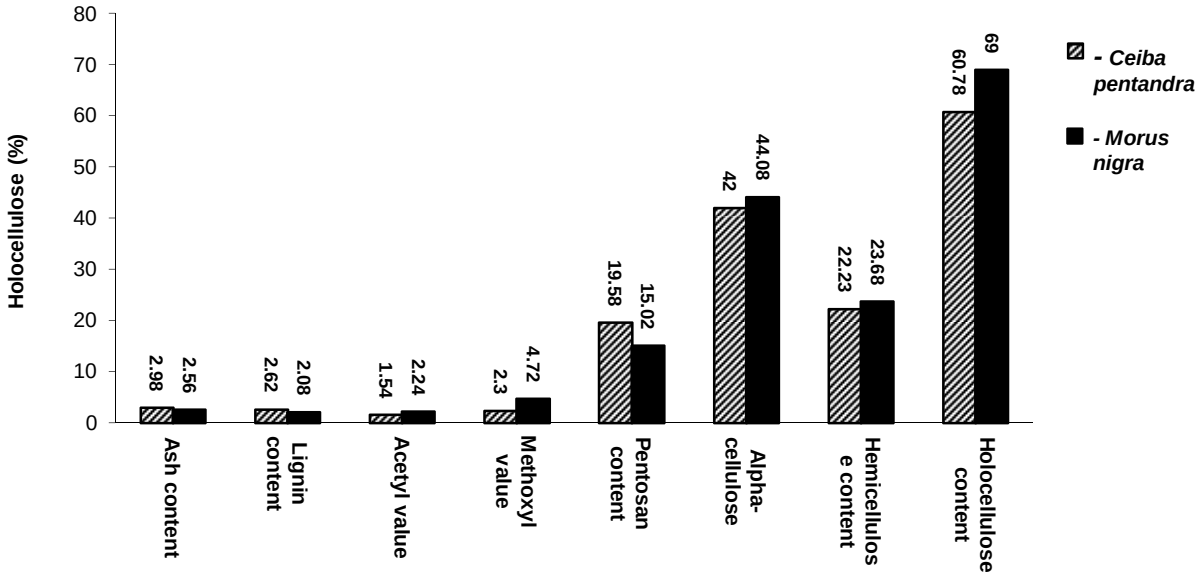

Figure 1: Comparative study of chemical analysis of holocellulose.

The complete fractional amount of hemicelluloses in crude and purified yield is recorded in Table 2 and data are compared graphically in Figure 2.

Table 4.4: Fractional amounts of hemicelluloses.

Sr. No.	Fractions	*Ceiba pentandra*		*Morus nigra*	
		Crude (gm)	Purified (gm)	Crude (gm)	Purified (gm)
1.	5% KOH fraction (First extraction)	19.8945	18.3823	20.7215	19.2205
2.	16% KOH fraction (Second fraction)	5.2010	4.9905	5.9415	5.3028
3.	24% KOH+ 4% boric acid (Third extraction)	0.6202	0.5023	0.6710	0.5416
4.	Cellulose as final residue	42.26	-	44.98	-

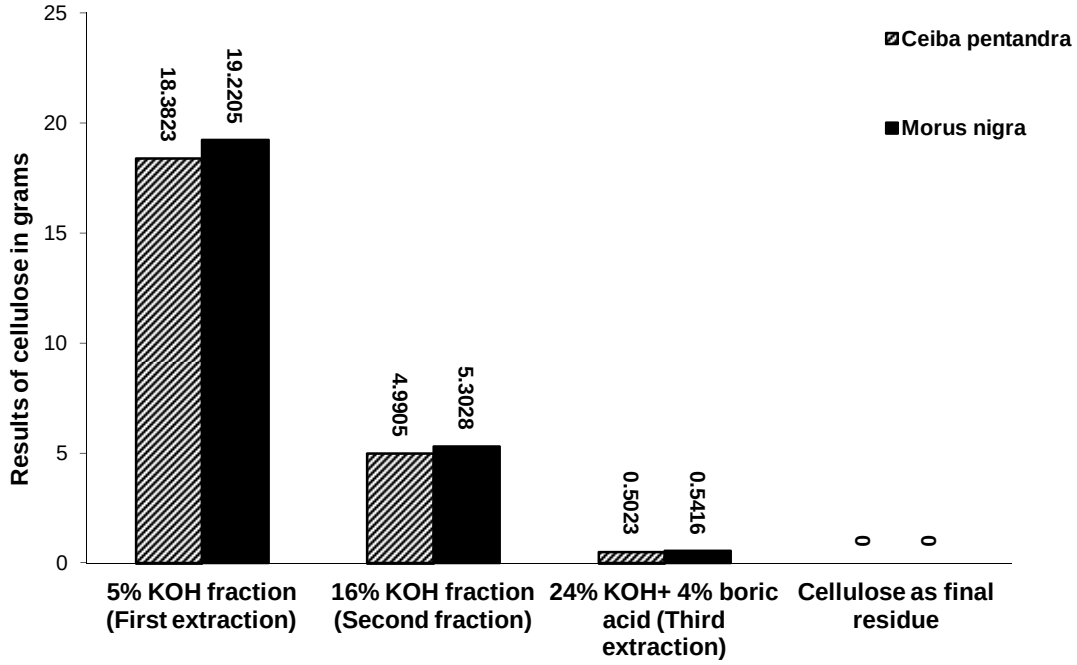

Figure 2: Fractional amounts of hemicelluloses.

From the critical analysis of observations tabulated in the Table 2 of chemical analysis of holocellulose the following results are very clear. The values of ash content, lignin content and pentosans content of *Ceiba pentandra* are slightly higher than values of *Morus nigra.* Whereas acetyl, methoxyl value, alpha-cellulose, hemicellulose and holocellulose contents are higher in *Morus nigra* as compare to *Ceiba pentandra*. The comparison of the percentage of the above various contents in holocellulose of both the samples show that the percentages of all the above contents are higher in *Morus nigra*. The above data also confirmed that during the course of extraction of holocellulose, easy delignification and more degradation of the carbohydrate fraction take place. These results also confirm that the hemicellulose governs the pore size in the cell wall and thus the rate of delignification. As a result removal of hemicellulose increases the pore size as well as the rate of delignification. The present results are in

agreement with the findings of Kerr et. al [12], Cunningham et al.[6], Bhat et al.[1], Montier et al.[17], Shimizu[25] and Laine [13].

From the analysis of the results of fractional amounts of hemicelluloses (Table 2) it is revealed that more amount of hemicellulose is obtained by 5% KOH extraction than by the 16% KOH and mixture of 24% KOH (4% boric acid) extraction and little more amount of hemicellulose is obtained by 16% KOH alone than with mixture of 24% KOH (4% Boric acid) extraction. It is also clear with this observation that increasing the percentage of alkali reduces the values of hemicellulose. This trend is true for both the *Ceiba pentandra* and *Morus nigra* samples. During the first, second and third KOH extraction the amount of purified hemicellulose is more in *Morus nigra* as compared to *Ceiba pentandra*. It should be stressed that while the number of steps discussed in the sequence are reduced, unchanged hemicelluloses are not obtained during alkaline extractions. The present observation shows similarities with the findings of Poddar [21], Shukla[26], Harmov et al.[10], Scherz and Bonn [22] and Essam and Sayed-el [9].

During the isolation, extraction and purification of hemicelluloses in the present study of both the samples of *Ceiba pentandra* and *Morus nigra*, crystallization did not occur in the hemicellulose fraction. This simply indicates that the hemicelluloses are amorphous.

CONCLUSION

From the observed results and their discussions, it may be concluded that during the course of extraction / chemical analysis of holocellulose, easy delignification and more degradation of the carbohydrate fraction take place and the removal of hemicellulose increase the rate of delignification. The preliminary fractional amounts of hemicelluloses during the extraction process are to obtain the galactoglucomannan series in practically pure from. It is also concluded that by using the alkaline extracted fraction, the hemicelluloses are obtained in pure form and hemicellulose fraction did not show crystallization which indicative of its amorphous nature.

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