

## THE RELATIONSHIP BETWEEN TWO METHODS FOR EVALUATING FIVE-CARBON SUGARS IN EUCALYPTUS EXTRACTION LIQUOR

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Alkaline pre-extraction and hydrothermal hydrolysis were carried out with eucalyptus chips. Two methods were used to determine and calculate the extraction yield of five-carbon sugars. One is an indirect method, based on the difference of pentosan content between the chips before and after pre-extraction. The other method is to directly measure the content of five-carbon sugars in the extracting solution. The results indicated that there was a defined relationship between the two methods. For alkaline pre-extraction, a good logarithmic relationship was shown. There was a good linear relationship for hot water prehydrolysis. So the pentosan content of the extracted chips could be predicted from the results of the latter method, referring to the two relational expressions. In this study, a simple and rapid method of spectrophotometry was introduced, which will help in the evaluation of extraction yield of five-carbon sugars during biomass processing.

*Keywords:* Eucalyptus; Five-carbon sugars; Spectrophotometric method; TAPPI Standard method; Alkaline pre-extraction; Hydrothermal hydrolysis

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### INTRODUCTION

As one of the most important components in plant cell walls, hemicellulose shows great differences in content and structure among different kinds of plant fibers. In general, the main hemicellulose in hardwood species is xylan, accounting for about 20% to 30% of the dry mass. Eucalyptus is a fast growing hardwood species, which is an important raw material in the pulping and papermaking industry. So the determination of pentosan is significant for evaluating hemicellulose in eucalyptus chips. Recently, related research has been focused on the integration of biorefinery and pulping (Van Heiningen 2006), of which hemicellulose pre-extraction is an important part. Thus, it is useful to develop a rapid method to measure five-carbon sugars in the extraction liquor.

In the pulp and paper industry the pentosan content in wood and pulp is usually determined by a TAPPI Standard method (TAPPI Standard T223 1984). The TAPPI method requires that the chips should be ground into wood meal, followed by moisture equilibration and measurement. The more precise quantitative method is based on chromatography. Samples are hydrolyzed into monosaccharides, and the pentosans are quantified as the sum of xylose and arabinose, which are separated by liquid or gas

chromatography (Kitayama et al. 2000). Both methods are popular, but the sample preparation procedures are often complex and time-consuming. Additionally, the chromatography requires high costs of sugar columns and instrument maintenance. Although some colorimetric methods such as the 3,5-dinitrosalicylic acid (DNS) assay and the phenol-sulfuric acid method could be used to quantify total reducing sugars, they are poor in measurement accuracy and reliability (Miller 1959; Dubois et al. 1956). In the food industry, Douglas method has been used for rapid determination of pentosan in food effluent (Cerning and Guibot 1972; Douglas 1981). Yu et al. (2007) established the feasibility of measuring pentosan in the Eucalyptus extraction liquor by this method, including an optimization for the conditions used in this method.

In the present study, eucalyptus chips were used for alkaline and autohydrolysis pretreatments. Spectrophotometry (Yu et al. 2007) and the TAPPI standard method were used for analyzing pentosan content in the extraction liquor and wood chips, respectively. The main purpose was to investigate the relationship between the two methods, so that the pentosan content of extracted wood chips could be calculated by the rapid and simple colorimetric method.

## EXPERIMENTAL

### Materials and Chemicals

The eucalyptus chips (*Eucalyptus ABL 12*) were obtained from a pulp mill located in Guangdong province, China. The analytical results of wood components are listed in Table 1.

Chemicals used in the experiment were of analytical grade, except xylose (biochemical grade), and all of them were obtained from commercial sources.

**Table 1.** Chemical Components of Eucalyptus Chips

Chemical Components	Content (%)	Chemical Components	Content (%)
Cold Water Extractive	1.61	Pentosan	20.64
Hot Water Extractive	2.72	Acid Soluble Lignin	5.79
1% NaOH Extractive	13.25	Klason Lignin	28.04
Benzene-Ethanol Extractive	0.70	Ash	0.33
Nitric Acid-Ethanol Cellulose	46.46		

### Pretreatment and Washing of Wood Chips

The wood chips were pre-extracted by two methods: alkaline pre-extraction and hydrothermal hydrolysis. Pretreatment was conducted in a water bath (below 100°C) or in an autoclave (above 100°C). The conditions were as follows: temperature 90~180°C, alkaline concentration 1~14%, time to temperature 0~60min, time at temperature 30~480 min, and liquor to wood ratio 1:5~1:10.

After pretreatment, the filtrate was separated and collected for analysis. For alkaline pre-extraction, the chips were washed repeatedly and then soaked in distilled water until the washing liquor was colorless, then air dried. For autohydrolysis, the chips were washed twice with distilled water and air dried immediately. No sugar analysis was performed on the washing liquor due to lower sugar concentration.

### Preparation of KP Lignin and Acid Precipitate

Kraft lignin in the black liquor and acid precipitate from the alkaline extraction solution were prepared by acid precipitation. The cooking conditions for Kraft pulping were: alkaline charge 20%, sulfidity 30%, maximum temperature 165 °C, H-factor 1300, and liquor to wood ratio 4:1. The conditions for alkaline pre-extraction were: alkaline concentration 10%, liquor to wood ratio 10:1, 90 °C, and 2 hours.

In the precipitation process, 30% sulfuric acid was added slowly into black liquor or alkaline extraction liquor at 60 °C with stirring. The mixture was adjusted to a pH of 3, and the resulting precipitate was separated by centrifugation, which was then washed with distilled water and vacuum dried. The kraft lignin sample was purified according to Lundquist and Kirk (1980), while there was no purification for the acid precipitate from alkaline extraction liquor. The two samples were named PL and UPL in turn. Their solutions were made by dissolving them in dilute alkaline solution.

### Determination and Calculation of C5 Sugar Yield in Wood Chips

The initial and extracted wood chips were air dried and milled, and screenings between 40 and 60 mesh were accepted for study. The pentosan content in wood chips was determined by TAPPI Method T223 (1984), and the extraction yield of five-carbon sugars was calculated with Eq. (1),

$$P_s = \frac{(S - Y)}{S} \times 100\% \quad (1)$$

where  $P_s$  is the yield of C5 sugars (%), TAPPI Standard method), and  $S$  and  $Y$  are the pentosan content in the initial and extracted wood chips (% weight percent), respectively. The pentosan content of initial chips in this research was 20.64%.

### Determination and Calculation of C5 Sugar Yield in Extraction Liquor

The principle of the colorimetric method is that at high temperature in acidic medium, the pentosan is hydrolyzed into pentose, which dehydrates into furfural and then creates colored products by reacting with phloroglucinol. Owing to its absorption at the specific wavelength of 553nm, the content of five-carbon sugars can be determined, giving the C5 sugar yield (Douglas 1981).

2 mL of liquor sample was mixed with 10mL of color reagent (2 g phloroglucinol, 110 mL glacial acetic acid, 10mL anhydrous ethanol, 2 mL hydrochloric acid and 1mL 17.5g/L glucose solution were mixed thoroughly). The color reaction was processed in a 100°C water bath for 10 minutes, and the absorbance at 553 nm of the resulting solution was recorded. For the detailed operating procedure, see reference (Yu et al. 2007). The yield of C5 sugars was related to the pentosan content in the initial wood chips and the spectrophotometric results of C5 sugars in the extraction liquor, as shown in Eq. (2). While the equation is derived from two different analytical methods, it should still be useful in expressing the final dissolving trend.

$$P_R = \frac{L \times C \times n \times 10^{-6} \times 0.88}{S}, \quad (2)$$

where  $P_R$  is the relative yield of C5 sugars;  $L$  is liquor to wood ratio (mL/g);  $C$  is xylose concentration ( $\mu\text{g/mL}$ ) from spectrophotometric method;  $n$  is dilution factor;  $S$  is the same as in Eq. (1), and 0.88 is the polymerization factor from pentose to pentosan, namely,  $\text{Pentosan content} = \text{Pentose content} \times 0.88$  (TAPPI Standard T223 1984).

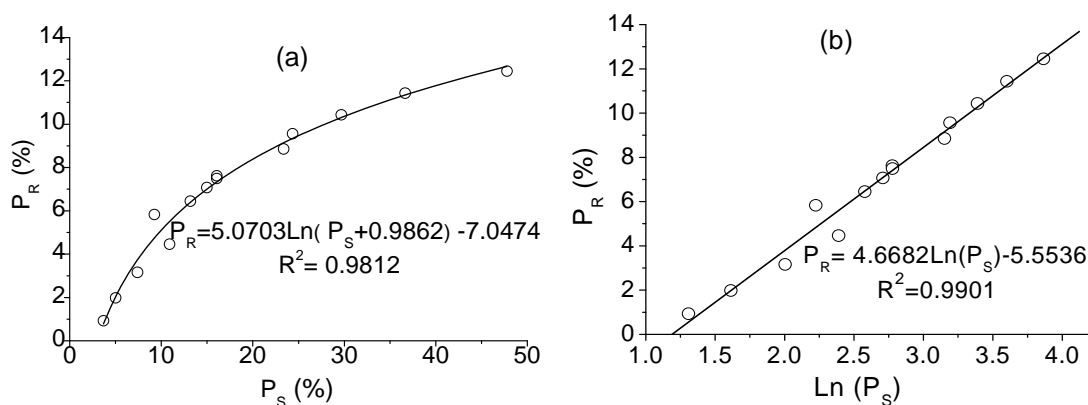
## RESULTS AND DISCUSSION

Compared with the usual time-consuming operation of measuring pentosan content in wood chips, the spectrophotometric method is a simple and rapid technique to measure five-carbon sugars in the extraction liquor. Thus, the relationship between the two methods was investigated, then the extraction results could be expressed with this rapid method. The chips were pre-extracted with alkaline solution or hot water under different conditions. Then the yield of C5 sugars could be calculated by Eq. (1) and Eq. (2), so that the relationship between the two methods could be concluded from the two groups of data.

### Relationship between the Two Methods for Alkaline Pre-extraction

Alkaline pre-extraction was done at 90 °C with different alkaline concentration for different time, and the C5 sugar yield ( $P_S$ ) was in the range of 3.7% to 47.8%. The results calculated from Eq. (1) and Eq. (2) are shown in Fig. 1(a). A logarithmic relation was found between  $P_S$  (TAPPI Standard method) and  $P_R$  (Spectrophotometric method) in Fig. 1(a), which was verified in Fig. 1(b). Obviously, there was a good linear relationship between  $P_R$  and  $\ln(P_S)$  (Eq. (3)).

$$P_R = 4.6682 \ln(P_S) - 5.5536 \quad (R^2 = 0.9901) \quad (3)$$



**Fig.1.** Relationship between the two methods for alkaline pre-extraction

Consequently, in the range of five-carbon sugar yield in this study, the real yield value ( $P_S$ ) could be calculated by Eq. (3) with the results of the rapid spectrophotometric method. Compared with the TAPPI Standard method, the experimental procedure was greatly simplified, which can be expected to result in improved work efficiency during routine analysis.

### Relationship between the Two Methods for Hydrothermal Hydrolysis

Hydrothermal hydrolysis was done at different temperature for different time, and the yield of C5 sugars ( $P_S$ ) was in the range of 2.1% to 75.1%. The results from Eq. (1) and Eq. (2) are displayed in Fig. 2. As can be seen from Fig. 2, a good linear relationship between the two methods was obtained, which was expressed with Eq. (4).

$$P_R = 0.5612P_S - 1.2086 \quad (R^2 = 0.9872) \quad (4)$$

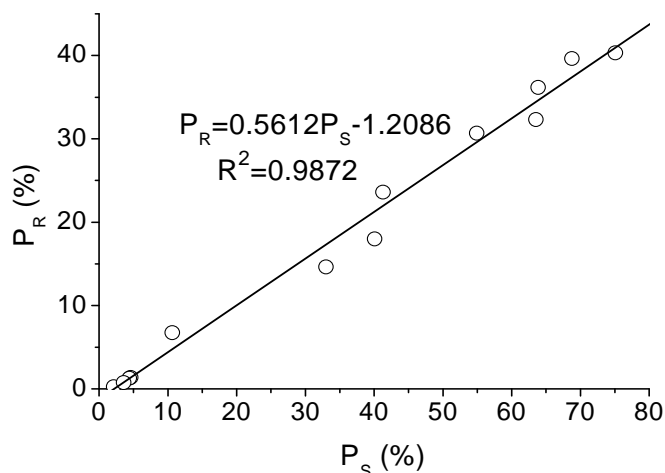
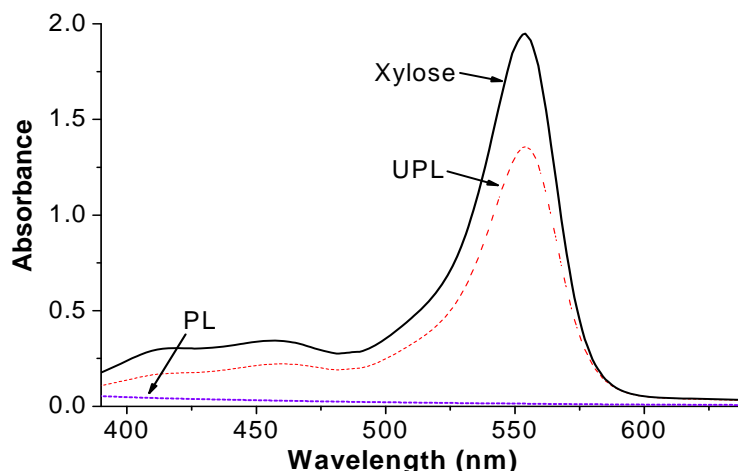


Fig. 2. Relationship between the two methods for hydrothermal hydrolysis

### Comparative Analysis between Different Pre-extraction Methods

The above results indicate that different relationships are appropriate for different extracting methods of alkaline pre-extraction and hydrothermal hydrolysis. One of the probable reasons for that is related to the washing and soaking of alkaline extracted wood chips before pentosan analysis. During alkaline pre-extraction, the pentosan in wood chips and the oligomers or monomers of five-carbon sugars resulting from alkaline degradation could be partially dissolved into the liquor (filtrate). However, a considerable amount of them remained in the chips. Subsequently, those remaining pentosans could gradually diffuse and become dissolved into the liquor during the following washing and soaking, which will lead to lower pentosan results in the extracted wood chips. Thus the difference of determined pentosan content between the initial chips and the extracted chips, as S-Y in Eq. (1), is relatively higher. Accordingly, relative higher yield of C5 sugars ( $P_S$ ) will result.

On the other side, acidic condition is needed for the color reaction, so the alkaline extraction liquor should be neutralized before analysis. Then part of lignin and Lignin-Carbohydrate Complex (LCC) is certain to be precipitated, which will lead to sugar loss in the filtrate and finally result in lower results for the colorimetric method. This presumption could be verified by the spectrum in Fig. 3.



**Fig. 3.** Visible spectrum of the color derivatives of xylose and interfering substances (The xylose concentration is 0.45g/L and the concentration of UPL and PL is 0.5g/L)

Apparently, the color derivative of purified Kraft lignin (PL) had no absorption at 553nm, which verifies that lignin did not interfere with the determination of pentose. But for the color derivative of acid precipitate (UPL) from alkaline extraction liquor, there was significant absorption at 553nm, which may have been the result of five-carbon sugars in LCC. These precipitated substances would not be detected in the spectrophotometric determination for the filtrate. So the determined content of C5 sugars in the alkaline extraction liquor was lower than the real result, and this difference may go up with the increasing concentration of C5 sugars in the extracting liquor.

Although no linear relationship was shown between the two methods for alkaline pre-extraction, the rapid spectrophotometric method and Eq. (3) can still be used for measuring the relative pentosan yield in the extracted wood chips. The study results will provide reference for future research related to biomass processing.

## CONCLUSIONS

1. Two different methods were used in this study. A TAPPI Standard method was used to analyze the pentosan content in the chips, while a spectrophotometric method was used for determining the five-carbon sugars in the extraction liquor.
2. For alkaline pre-extraction, a linear relationship was shown between the results of the spectrophotometric method and the natural logarithm of the result from the TAPPI method, which can be expressed as  $P_R = 4.6682 \ln(P_S) - 5.5536$  ( $R^2=0.9901$ ). For hydrothermal hydrolysis, there was a good linear relationship and the expression was  $P_R = 0.5612 P_S - 1.2086$  ( $R^2=0.9872$ ). It was verified that the two equations can be used for the evaluation of C5 sugars yield in the eucalyptus pre-extraction process with alkaline solution or hot water.
3. There are two probable causes of the different relationships for the two different extracting methods. One is washing and soaking of alkaline extracted wood chips, and the other is the acid precipitation of alkaline extraction liquor (filtrate). More investigation should be performed to further clarify these issues.

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## REFERENCES CITED

- Cerning, J., and Guilbot, A. (1972). "A specific for determination of cereals and cereal products," *Cereal Chemistry* 23(1), 177-184.
- Douglas, S. G. (1981). "A rapid method for the determination of pentosans in wheat flour," *Food Chemistry* 7(2), 139-145.
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956). "Colorimetric method for determination of sugars and related substances," *Analytical Chemistry* 28(3), 350-356.
- Kitayama, T., Morimoto, M., and Takatani, M. (2000). "Isolation of hemicellulose from a sorghum, *Andropogon sorghum* Brot, Kumadake no. 263 and determination of its constituent sugars," *Carbohydrate Research* 325(3), 230-232.
- Lundquist, K., and Kirk, T. K. (1980). "Fractionation-purification of an industrial kraft lignin," *Tappi Journal* 63(1), 80-88.
- Miller, G. L. (1959). "Use of dinitrosalicylic acid reagent for determination of reducing sugar," *Analytical Chemistry* 31(3), 426-428.
- TAPPI Standard T223 (1984). "Pentosans in wood and pulp."
- Van Heiningen, A. (2006). "Converting a kraft pulp mill into an integrated forest products biorefinery," *Pulp & Paper Canada* 107 (6), 38-43.
- Yu, J., Zhang, Z., and Chi, C. (2007). "An inquiry into a quick determination method for the pentosan in hemicellulose pre-extraction liquor from eucalyptus chips," *China Pulp and Paper* 25(11), 10-13.

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