Inter-laboratory comparisons of hexenuronic acid measurements in kraft eucalyptus pulps using a UV-Vis spectroscopic method

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ABSTRACT: An inter-laboratory comparison of a UV-Vis spectroscopic method (TAPPI T 282 om-13 "Hexeneuronic acid content of chemical pulp") for hexeneuronic acid measurements was conducted using three eucalyptus kraft pulps. The pulp samples were produced in a laboratory at kappa numbers of approximately 14, 20, and 35. The hexeneuronic acid contents of the three pulps were approximately 55–65 µmol/g, or with a variation of approximately 15%. Five laboratories from four different countries participated in this round-robin study as part of the evaluation of the TAPPI provisional method for upgrading to a TAPPI standard method. The comparative study showed that the orders of hexeneuronic acid content from low to high among the three pulp samples produced by four laboratories were in agreement. Replicate measurements were not conducted at the laboratory that produced an inconsistent order of hexeneuronic acid among the three pulp samples compared with the other four laboratories. The differences in hexeneuronic acid contents from different laboratories were systematic and consistent; that is, some laboratories consistently produced high values of hexeneuronic acid for all three samples. The comparative data of three pulp samples concluded that the measurement repeatability (within a laboratory) was less than 3% and reproducibility (among laboratories) was less than 16%.

Application: Analytical test laboratories, pulp mills, and research organizations can use the information in this paper for chemical pulp analysis.

In alkaline pulping, hydroxide ions react with 4-*O*-Me-glucuronic acid groups through the alkali-catalyzed reactions to form hexenuronic acids (HexA) [1]. The interest in determination of HexA in chemical pulps lies in the fact that HexA can greatly affect subsequent pulp bleaching operations [2]. For example, HexA can consume bleaching chemicals [3], bind with transition metals, and reduce pulp brightness [2]. The contribution of HexA to pulp kappa number was apparent from the kappa number measurements before and after removal of HexA through hydrolysis [4,5]. In enzymatic bleaching, the reduction in HexA content was observed to correlate with the reduction in the pulp's kappa number [6,7], suggesting that the application of enzymes to reduce HexA can help to reduce bleaching chemical consumption.

The determination of HexA has relied on hydrolysis of HexA and analysis of the hydrolysis products. Acid hydrolysis in conjunction with photometric measurement—the HUT method (from Helsinki University of Technology)—was applied [2]. Enzymatic hydrolysis using xylanase followed by nuclear magnetic resonance (NMR) spectroscopy—the VTT method (from the VTT Technical Research Centre of Finland)—was also evaluated [8]. The slow rate of acidic or enzymatic hydrolysis makes the HUT or VTT method very time consuming. Mercuric acetate was used to improve the hydrolysis efficiency in the KTH method (from the KTH Royal Institute of Technology, Sweden) [5]. The HexA was determined spectroscopically after periodate oxidation and the addition of thiobarbituric acid to form a red-colored chromophoric compound after high-performance liquid chromatography separation. However, the extra steps for spectroscopic determination make the KTH method just as tedious in quantifying the hydrolysis products. Furthermore, the potential yield loss from each chemical step can affect quantification accuracy of HexA. A comparative study was conducted using the HUT, VTT, and KTH methods [9]. The HUT method was found to produce consistently lower HexA values than the VTT and KTH methods for pulp samples with HexA >5 µmol/g. It is unclear whether the comparisons were made within the same laboratory or among different laboratories. Furthermore, the reproducibility of each of these three methods was not established. No conclusion was drawn on the superiority of the three methods.

A relatively simple and rapid method called the IPST method (from the Institutue of Paper Science and Technology; Atlanta, GA, USA) uses mercuric chloride ($HgCl_2$) for rapid

hydrolysis followed by direct spectroscopic quantification of the HexA hydrolysis product at 260 nm [10]. Spectral interference from leached lignin is corrected spectroscopically using a dual wavelength technique so that the method can be applied to unbleached pulps. This IPST method has attracted great interest and is now widely adopted by the scientific community around the world for its speed and convenience [6,7,11-19]. A TAPPI provisional method (T 282 pm-07 "Hexeneuronic acid content of chemical pulp") was developed based on this technique. This work is part of the TAPPI round-robin study for upgrading the provisional method to a TAPPI standard method (T 282 om-13 "Hexeneuronic acid content of chemical pulp"). This round-robin study included five laboratories from four countries—two laboratories in the United States and one each in Canada, China, and Spain. Three of the laboratories belong to research institutions, one is a commercial analytical laboratory, and one is a pulp mill.

MATERIALS AND METHODS

Chemicals and reagent

Mercuric chloride ($HgCl_2$) and sodium acetate trihydrate ($CH_3COONa•3H_2O$) were from commercial sources and were of analytical grade.

Laboratory	Spectrophotometer
Lab-1	DR5000 (Hach*)
Lab-2	Model 8453 (Agilent Technologies**)
Lab-3	Evolution 600 (Thermo Scientific***)
Lab-4	Model 8453 (Agilent Technologies**)
Lab-5	DR5000 (Hach*)

* Hach; Loveland, CO, USA
** Agilent Technologies; Santa Clara, CA, USA
*** Thermo Fisher Scientific; Waltham, MA, USA

I. UV-Vis spectrophotometers used at the five laboratories.

Production of pulp samples

The eucalyptus wood chips were provided by a pulp mill located in Hainan Province, China. The kraft pulping processes were conducted with a laboratory-scale digester at South China University of Technology, Guangzhou, China. Three eucalyptus kraft pulps were produced using an alkali charge of 20% (as sodium hydroxide [NaOH]) and sulfidity of 25% at a maximum pulping temperature of 155°C, with ramping time to maximum temperature of approximately 90 min. The pulping liquor-to-wood (o.d. weight) ratio was 4:1. The kraft cooks were terminated after 90, 120, and 150 min at 155°C to obtain the desired pulps with different kappa numbers and HexA contents. The digester was cooled with tap water at the end of each cook. The cooked material was disintegrated and washed. The kappa numbers of the resulting three brownstocks were then determined using TAPPI T 236 om-06 "Kappa number of pulp." Pulp pads of approximately 600 g/m^2 were made and air dried to a moisture content of approximately 90% before distributing to the different laboratories.

Sample distribution

The pulp pads of three eucalyptus pulps produced at South China University of Technology were shipped to the USDA Forest Products Laboratory for distribution to the other three laboratories. Handsheets of each sample were shipped to Econotech in Canada, Verso Paper in Michigan, and Universitat Politècnica de Catalunya (BarcelonaTech) in Spain for analysis within a week.

Determination of HexA

Hydrolysis of HexA was conducted at each laboratory according to the method described by Chai et al [8]. Specifically, 6 g of $HgCl_2$ and 7 g of $CH_3COONa•3H_2O$ were dissolved in 500 mL of distilled water in a beaker. The solution was then transferred to a 1-L volumetric flask and diluted to

Kappa No.	Pulp-1			Pulp-2			Pulp-3		
	19.8			13.9			33.9		
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
w (g)	0.0500	0.0507	0.0495	0.0501	0.0505	0.0501	0.0493	0.0492	0.0495
A_{260}	**1.4767**	**1.5215**	**1.4905**	**1.3532**	**1.3986**	**1.4195**	**1.3894**	**1.3361**	**1.3530**
A_{290}	**0.2661**	**0.2797**	**0.2669**	**0.2474**	**0.2513**	**0.2676**	**0.3237**	**0.2508**	**0.2670**
HexA (μmol/g)	**66.44**	**66.59**	**67.91**	**60.46**	**62.34**	**62.87**	**58.24**	**60.33**	**59.87**
Mean HexA (μmol/g)	**67.0**			**61.9**			**59.5**		
Standard deviation (μmol/g)	**0.81**			**1.26**			**1.10**		
Relative standard deviation (%)	**1.21**			**2.04**			**1.85**		

II. Experimental repeatability at Lab-2.

0.6% $HgCl_2$ and 0.7% CH_3COONa using distilled water. A sample weight of 0.05 g of pulp with known moisture content was added to a 20-mL vial containing 10 mL of the 0.6% $HgCl_2$ hydrolysis solution. The vial was shaken by hand to mix the chemicals with the sample after sealing the vial with a septum. Experiments using 0.5 g of pulp were also conducted at one laboratory to study the effect of pulp suspension consistency on HexA determination. The vial was heated for approximately 30 min in a water bath at 60°C-70°C. The resulting solution was retrieved using a 3-mL plastic syringe after cooling the vial with tap water to room temperature. The retrieved solution was filtered using a 0.2-µm syringe filter attached to the plastic syringe to filter fines before dispensing the filtrate into a 10-mm path length silica cuvette for UV absorption measurements on a commercial UV spectrophotometer. The spectrophotometers used in the five laboratories are listed in **Table I**. The absorption signals were recorded at 260 and 290 nm. The HexA content was determined using Eq. (1):

$$C_{HexA}(\mu mol/g) = 0.287 \times \frac{(A_{260} - 1.2A_{290}) \cdot V(mL)}{w(g)} \quad (1)$$

where 0.287 is a calibration constant. This calibration constant is universally used to calculate the HexA content in any pulps, including the pulps evaluated in this study; the factor of 1.2 is used to correct for lignin absorption on HexA determination. Different lignin may have varied spectral absorption coefficients that need to be considered for measuring HexA of bleached pulps [20]; V is the volume of the hydrolysis solution in mL, and w is the weight of the o.d. pulp sample used in the hydrolysis, in grams.

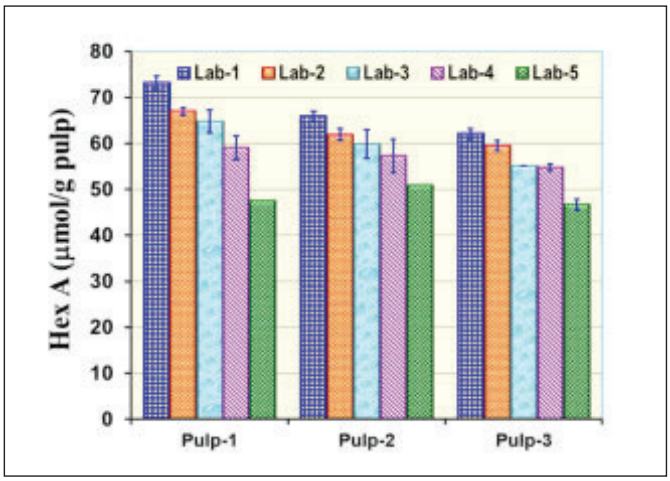
RESULTS AND DISCUSSION

Experimental repeatability—within-laboratory measurement

The kappa numbers of the three pulp samples were measured at South China University of Technology and are listed in **Table II**. The five laboratories are labeled as Lab-1, Lab-2, Lab-3, Lab-4, and Lab-5 in the data presentation. Replicate measurements were conducted at Lab-1, Lab-3, Lab-4, and Lab-5 (only one sample) while triplicate measurements were conducted at Lab-2. The triplicate data from Lab-2 are listed in Table II to demonstrate the experimental repeatability. The results indicate excellent repeatability with a maximum relative standard deviation (RSD) of approximately 2%. Similar repeatability was obtained from the other laboratories. The results also indicated that Pulp-1 had the highest HexA content while Pulp-3 had the lowest; however, the difference in HexA between Pulp-1 and Pulp-3 was very small at approximately 12%. It will be interesting to see whether the measurements from different laboratories can differentiate this small difference.

	Pulp-1	Pulp-2	Pulp-3
Lab-1	73.3	65.9	62.1
Lab-2	67.0	61.9	59.5
Lab-3	64.8	59.8	55.1
Lab-4	59	57.3	54.8
Lab-5	47.5	50.8	46.7
Standard deviation	**9.74**	**5.63**	**5.86**
Mean	**62.3**	**59.1**	**55.6**
Relative standard deviation (%)	**15.6**	**9.5**	**10.5**
0.5*(maximum within-laboratory standard deviation)	**1.27**	**1.6**	**0.57**
Standard deviation among laboratories	**9.82**	**5.85**	**5.86**
Reproducibility (%)	**15.7**	**9.9**	**10.6**

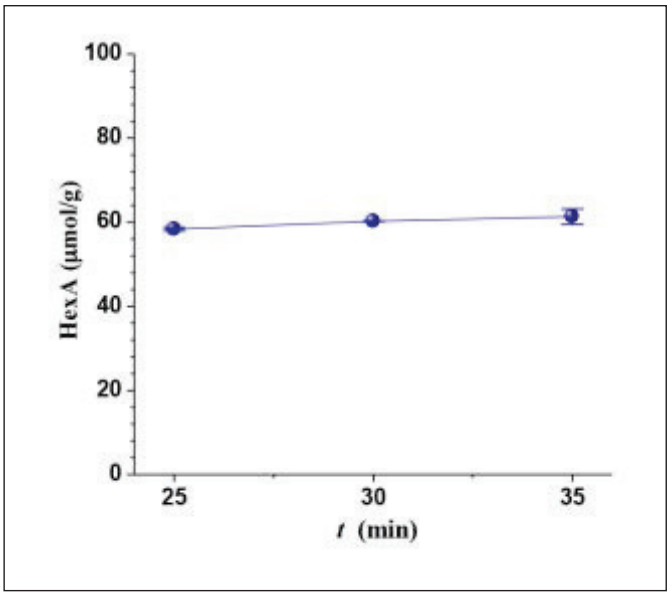
III. Experimental reproducibility: comparison of measured hexeneuronic acid (HexA, µmol/g) among five laboratories.



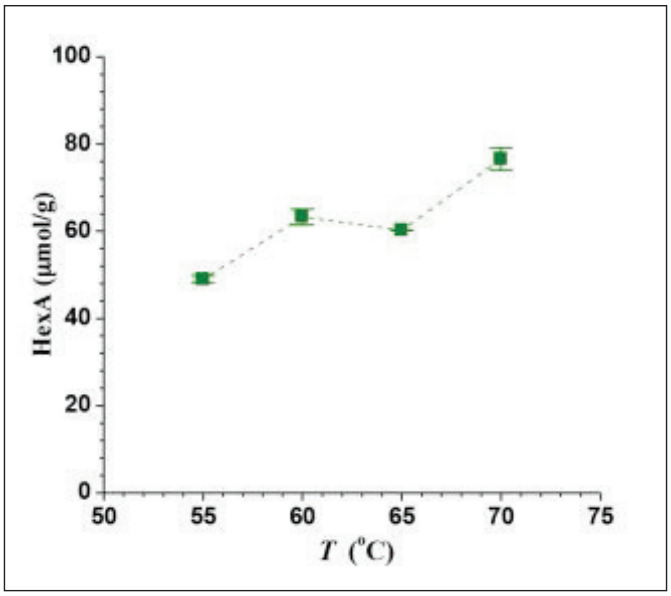
1. Mean HexA and standard deviations from the five laboratories.

Experimental reproducibility—among-laboratory measurements

The mean values of HexA of the three pulp samples measured by the five laboratories were compared (**Table III**). Both the mean value and standard deviation [SD] of the five laboratory measurements were calculated. The reproducibility refers to the among-laboratory variability. For a given sample, it can be defined as the deviation of the mean of individual laboratories from the global mean of the all five laboratories plus the half (duplicate) of the maximum deviation of within-laboratory measurement. The results indicated that the order of HexA content from low to high among the three pulp samples measured by four laboratories (Lab-1, Lab-2, Lab-3, and Lab-4)



2. Effect of hydrolysis duration on HexA.



3. Effect of hydrolysis temperature on HexA.

were consistent. Lab-5 conducted replicate measurements only for Pulp-3. No replicate measurement was made for Pulp-1, which had a lower value than Pulp-2 and Pulp-3; this is contrary to the results from the other four laboratories. To better illustrate this difference, the mean HexA and SD from the five laboratories are plotted (**Fig. 1**). The results clearly showed that the order of HexA from the four laboratories was consistent; that is, each laboratory (except for Lab-5) was able to differentiate between the three pulp samples even though the maximum difference among them was only 12%. Furthermore, the differences among the five laboratories are systematic; that is, the HexA contents for each pulp sample measured at Lab-1 were consistently higher than the corresponding values measured at Lab-2, Lab-3, and Lab-4, and Lab-5. This systematic difference was not believed to be due to a particular brand of spectrophotometer because the differences between Lab-1 and Lab-5, and between Lab-2 and Lab-4, were obvious even though the same model of spectrophotometers from the same manufactures were used (Table I). The systematic difference could be due to the variations in hydrolysis reaction at different laboratories using different reaction temperatures because the method specified a temperature between 60°C–70°C, as will be discussed later. It also could be due to measurement errors in moisture content of the samples.

The maximum RSD among the five laboratories was approximately 15% (Pulp-1). The maximum relative reproducibility among the five laboratories was approximately 16% (Pulp-1) that included half (due to duplicate hydrolysis experiments) of the maximum SD of the sample (Pulp-1).

Effect of pulp hydrolysis conditions on HexA measurement

A high pulp consistency needs to be used for testing samples with low HexA content to increase sensitivity. At Lab-4, the sample weight was increased 10 times to 0.5 g o.d. weight using the same amount of 10-mL hydrolysis solution (i.e., the solids consistency was 5%). The same handshaking procedure was applied for mixing without the application of shear mixing. The filtered hydrolysis solution was then diluted 20 times before UV absorption measurements to avoid saturation. The measured HexA content of the three pulp samples was found to be consistently lower than the corresponding value measured using a 0.05-g pulp sample, or 0.5% solids consistency (**Table IV**). This suggests that solids consistency does have an effect on pulp hydrolysis reactions due to lack of mixing at high solids consistency; however, the relative difference was very small, less than 6% for Pulp-3 and less than 1.5% for Pulp-1.

	Pulp-1	Pulp-2	Pulp-3
w (g/10 mL)	0.053	0.051	0.053
Mean HexA	59.0	57.3	54.8
w (g/10 mL)	0.507	0.503	0.503
Mean HexA	58.9	56.1	51.6
Relative difference (%)	**1.5**	**2.2**	**6.1**

IV. Effect of hydrolysis consistency on measured HexA (µmol/g) at Lab-4.

The effects of hydrolysis temperature and duration on HexA measurements were also evaluated at Lab-4 using Pulp-3. The results suggest that hydrolysis duration has minimal effect on HexA, as shown in **Fig. 2**. However, the final HexA value is affected by hydrolysis temperature, as shown in **Fig. 3**. We suggest that hydrolysis should be conducted at 65°C for 30 min. **TJ**

CONCLUSIONS

This inter-laboratory comparison of HexA content measurement using the TAPPI provisional method T282 om-13 indicated the method is repeatable within laboratory analysis. The method is also reproducible based on inter-laboratory measurements. The method is very simple and rapid compared to all published methods.

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ABOUT THE AUTHORS

We chose to conduct this research because HexA content of chemical pulps affects optimal bleaching operation. This inter-laboratory comparison further validates the rapid method for HexA analysis we developed previously and provides guidelines for achieving good measurements.

Coordination among five laboratories in four different countries is very challenging. We just needed to be patient and organized and work with lots of good people.

The good repeatability and reproducibility of the method were very rewarding. Mills can use this method to significantly speed up chemical pulp analyses.

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