

Adaptation of the Jayme-Wise procedure for determination of principal components of fibre in sugar-cane varieties

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ABSTRACT

Attempts were made to adapt the Jayme-Wise procedure for the determination of α -cellulose, hemicellulose and lignin in woody plant tissue for softer sugar-cane fibre (bagasse) to facilitate increased sample through-put. Recoveries of hemicellulose were generally within the expected range of 23-32% using alkaline hydrolysis regimes of 24 hr at 4 °C and 1.25 h at 26 °C. Yields of α -cellulose varied from 27-35.3%. Several of these were well within the reported range of 29.2-32.4%. Lignin recoveries ranged from 6.18-35.3 % compared to the expected range of 18-24%. An increase in the delignification period from 5 hr to 24 h resulted in a notable increase in lignin levels. It would appear that loss of holocellulose occurred during separation from lignin, causing an enhancement of lignin levels. This enhancement appears to have exerted a greater negative effect on α -cellulose recovery compared to hemicellulose recovery. An examination of alternative sample handling and separation procedures for holocellulose, in particular and perhaps to a lesser extent for α -cellulose to reduce losses is critical to further adaptation of the Jayme-Wise procedure. Use of reference materials and reference methods would also be useful for quality assurance.

Keywords: bagasse, fuel, energy

INTRODUCTION

Optimising the energy potential of sugar-cane is one of several options that can be explored in the diversification of any sugar industry for economic viability. Such a strategy enables enhanced co-generation to a level that would facilitate the sale of electricity to the national grid and, reduce the industry's dependence on fossil fuel during the out-of crop season. Projects of this nature often qualify for carbon credits owing to a measurable reduction of greenhouse gases.

The ignition and combustion properties of bagasse, commonly used as fuel during the crop seasons, are major considerations in energy potential maximization. These are a function of fibre content and composition, ash content and moisture content. The fibre content of conventional sugar-cane is typically 11-16%. This compares with 13-25% for the newly developed multipurpose cane (MPC) and fuel cane varieties (Rao, 2004) The fibre composition of bagasse is dependent on climate, soil type, variety, age of cane at harvest, harvesting method, extent of cane washing and factory milling efficiency. The organic component of bagasse consists mainly of cellulose (32-48%), α -cellulose (29.9-32.4%), hemicellulose (23-32%), lignin (18-24%) [Han and Rowell, 1997 and Nordin, 1994]. Combustion is enhanced by the formation of heat generated when flammable volatile compounds produced at temperatures in excess of 200 °C mix with air (Schniewind, 1989). The continuous production of heat from these exothermic reactions propagates combustion. Cellulose produces the highest, and lignin the lowest level of flammable volatile compounds. The higher the cellulose content of bagasse the higher the combustibility it may be expected to have.

The α -cellulose, hemicellulose and lignin contents of woody materials are commonly determined by the Jayme-Wise method (MacFarlane et al, 1999, Laevitt and Danzer, 1993 and Green, 1963). The method involves Soxhlet extraction to remove soluble organic compounds (waxes, oils and resins) referred to as extractives, followed by extraction of lignin from the insoluble extractive-free material using sodium chlorite and acetic acid to produce holocellulose, then alkaline hydrolysis of holocellulose to yield insoluble α -cellulose and soluble hemicellulose. Holocellulose is the carbohydrate fraction of plant material that is insoluble in water (Han and Rowell, 1997). Various modifications of the Jayme-Wise method have been made to reduce the amount of glassware and sample required for processing (Laevitt and Danzer, 1993 and Green, 1963). The procedure however still remains tedious, lengthy and at risk for sample loss. Given the fact that the method was originally developed for woody material, there is clearly potential for shortening the processing times of various steps for bagasse which is less woody, without compromising data reliability in order to increase sample throughput.

This research was conducted to adapt the Jayme-Wise method to the determination of α -cellulose, hemicellulose and lignin contents of sugarcane bagasse from different sources. The analyses were conducted on a composite sample of commercial cane delivered to, the factory and laboratory samples of two (2) distinct cane varieties. The composite was selected from knifed cane carrier at the factory, while the remaining fibre samples were prepared with a Jeffco Cutter-Grinder followed extraction of free juice by a Carver Press. During the study, the method of sample presentation for Soxhlet extraction, and the duration of Soxhlet extraction, delignification and alkaline hydrolysis were varied.

EXPERIMENTAL PROCEDURE

Extraction

Samples (5-6 g) of oven-dried un-ground knifed (composite) cane and oven-dried, ground knifed (composite) cane were weighed (± 0.1 mg) on filter paper and in draw-string brown cotton bags respectively. The un-ground knifed cane samples were transferred to the Soxhlet extractors, which were plugged with pre-weighed glass wool to immobilise the samples. The ground samples were transferred to the extractors in the brown cotton bags after they were moistened with 5 mL of ethanol to minimise loss during transferral. Extractions were carried out in duplicate; there were 2 x 1 un-ground knifed samples per extractor and two (2) ground, knifed bagged samples per extractor. Extractions were carried out using a mixture of 200 mL toluene and 100 mL ethanol in a 1 L round-bottom distillation flask containing 3-4 large boiling chips. Extractions were conducted in a fume hood, and extraction times were varied from 8-24 hr. The sugar-cane varieties, DB 7869 (the company's standard variety) and WI 81456 (a potential fuel cane variety) were extracted in bags with extraction times of 12 hours. Extractions were carried out in duplicate as per the ground, knifed samples.

After cooling, each extractive-free un-ground sample was transferred to a pre-weighed filter paper placed in a Buchner funnel. The glass wool plug was also transferred to the funnel and washed with distilled deionised water to remove any residual material. The Soxhlet extractor was rinsed with distilled deionised water, and the washings transferred to the funnel. The sample was washed with 500 mL distilled deionised water, vacuum filtered, then air-dried (in fume hood). The sample was then oven-dried at 70 °C to constant weight.

Each extractive-free ground sample was squeezed dry of extractant using tweezers. The bag was then transferred to a funnel resting in the conical flask, the draw-string was loosened and the sample was left to air-dry for one hour in a fume hood. The sample was rinsed with 4 x 50 mL aliquots of ethanol, then left to air-dry overnight. The sample was then removed from the bag and transferred to the conical flask via the funnel. The bag was turned inside out and as much of the residue as possible was also transferred to the funnel. The bag was placed in a pre-weighed petri dish with cover and oven-dried at 70 °C to constant weight. The sample was washed with 5 x 100 mL aliquots of distilled deionised water and vacuum filtered using a magnetic funnel containing a pre-weighed 47 mm GF/A filter paper. The residue and filter paper were transferred to a pre-weighed aluminium dish with cover, air-dried in a fume hood for one hour, then oven-dried at 70 °C to constant weight to give the amount of extractive-free material.

Delignification

The unground extractive-free material was transferred to a 250 mL flat bottom distillation flask using tweezers. The filter paper was weighed to determine the mass of any remaining extractive-free sample. One percent (1%; w/v) sodium chlorite was added to the sample to give a reagent/sample (v/w) ratio of 40 mL/g, followed by 1 mL of glacial acetic acid and a magnet. The flask was then heated at a temperature of 70-75 °C in a water bath. Additional glacial acetic acid was added to the unground extractive-free sample to maintain a pH of 4, and the sample was stirred every 15-30 minutes. The reaction was allowed to continue for 5 hours at which time the sample (holocellulose)

exhibited a pale yellow colour. This procedure was repeated using an increased reaction time of 24 hours. In each case, the flask was cooled and the sample vacuum-filtered using a pre-weighed 47 mm GF/A filter paper and a Buchner funnel. Soluble lignin was removed in the filtrate. The holocellulose residue was washed with 4 x 100 mL portions of cold distilled deionised water and again vacuum filtered. It was then transferred to a pre-weighed aluminium dish with cover and oven-dried at 70 °C to constant weight. Any material passing through the filter paper was recovered by vacuum filtration on another pre-weighed filter paper, which was transferred to a separate pre-weighed petri dish and oven-dried at 70 °C to constant weight.

The ground extractive-free sample (3-5 g \pm 0.1 mg) was similarly treated with 1% (w/v) sodium chlorite to give a reagent/sample (v/w) ratio of 40 mL/g, followed by 1 mL of glacial acetic acid in a 250 mL conical flask, which was covered with a watch glass (or small petri dish) to facilitate reflux for 24 h. During this period, 1.00 g sodium chlorite and 0.6 ml of glacial acetic acid were added to the sample at four one hourly intervals. The sample was stirred at hourly intervals. The sample exhibited a white translucent appearance at the end of the 24 h period. This procedure was repeated using reduced reaction times of 15 hours and 10 hours. The reaction mixture, which contained the soluble lignin, was decanted from the insoluble holocellulose. A 100 mL aliquot of cold distilled deionised water was added to the holocellulose, and the mixture was thoroughly mixed. The mixture was allowed to settle and the wash water decanted. This process was repeated three times. The holocellulose was then vacuum-filtered onto a pre-weighed 47 mm GF/A filter paper using a magnetic funnel. The filter paper and holocellulose were transferred to a pre-weighed aluminium dish with cover and oven dried at 70 °C to constant weight. Any material passing through the filter paper was recovered as described previously for holocellulose from the un-ground extractive-free sample. The lignin content was determined from the difference in weight between the dried extractive-free material and the dried holocellulose

Alkaline Hydrolysis

The dried holocellulose from the unground extractive-free material was transferred to a pre-weighed 250 mL conical flask. The aluminium dish and filter paper were weighed to determine the mass of any remaining holocellulose, and 17.5% sodium hydroxide was added to the sample to give a reagent/sample (v/w) ratio of 20 mL/g, after which it was refrigerated (T = 4 °C) for 24 hours. The sodium hydroxide was then decanted from the resultant α -cellulose. The α -cellulose was vacuum-filtered using a Buchner funnel while washing with distilled deionised water. The filtrate contained soluble hemicellulose. The α -cellulose was then subjected to treatment (in the funnel) with glacial acetic acid for 3 minutes to neutralise the sodium hydroxide. The acid was removed by vacuum filtration. The funnel was then filled with distilled deionised water and the filtration was continued. This step was repeated until the α -cellulose was free of acid as indicated by blue litmus paper. An additional 250 mL distilled deionised water was added to the α -cellulose, which was then vacuum-filtered. Any α -cellulose passing through the funnel was recovered by passing it through the α -cellulose mat (in the funnel) while washing with distilled deionised water. The α -cellulose was then transferred to a pre-weighed aluminium dish with cover and oven-dried at 100-105 °C to constant weight.

A 3 g (\pm 0.1 mg) portion of dried holocellulose from the un-ground extractive-free material was intimately mixed with a 15 mL aliquot of 17.5% sodium hydroxide in a 250 mL conical flask for 1 minute at room temperature (\sim 26 °C). Ten (10) mL more of the base was added, followed by stirring for 45 seconds using a magnetic stirrer. This step was repeated using another 10 mL aliquot of base with a stirring time of 15 seconds. The flask was then covered with a petri dish cover and allowed to stand for 3 minutes. An additional 10 mL of base was added, and the reaction mixture was stirred for 10 minutes. A final 30 mL of base was added in 10 mL portions after 2.5, 5.0 and 7.5 minutes while stirring. Stirring was discontinued after 10 minutes. The flask was covered with a petri dish cover and allowed to stand for 30 minutes. A 100 mL portion of distilled deionised water was then quickly added to the reaction mixture, which was then thoroughly stirred and allowed to stand for 30 minutes bringing the total contact time with the base to 75 minutes. This procedure was also applied to holocellulose derived from the ground extractive-free sample.

The base containing the soluble hemicellulose, was decanted from the insoluble α -cellulose, and 25 mL of 8.3% sodium hydroxide was added. The dilute base was decanted and the α -cellulose was mixed with 50 mL of distilled water. The washings were removed by vacuum filtration without the use of filter paper. This step was repeated four (4) times. The α -cellulose was treated with a 30 mL aliquot of 2.5 M acetic acid, which was then removed by vacuum filtration. The α -cellulose was then washed with 5 X 50 mL aliquots of distilled deionised water. Consecutive washings were removed by vacuum filtration. Washing was continued with 4 x 100 mL aliquots of distilled water and consecutive washings were again removed by vacuum filtration. The α -cellulose was then treated with 30 mL of 2 M acetic acid and allowed to stand for 5 minutes. The acetic acid was then removed by vacuum filtration. The α -cellulose was subjected to washing using 8 x 100 mL of distilled deionised water to render it free of acid as indicated by blue litmus paper. The α -cellulose was washed over into the funnel using distilled deionised water, vacuum-filtered dry, then transferred to a pre-weighed aluminium dish with cover and oven-dried at 100-105 °C to constant weight. The hemicellulose content was determined from the difference in weight between the dried holocellulose and the dried α -cellulose.

RESULTS AND DISCUSSION

Overall, the reproducibility of the yields of α -cellulose, hemicellulose and extractives increased after the first run of the trial sample (TSR1), which was a composite of several commercial varieties (**Table 1**). Lignin yields were the least reproducible, while yields of extractives showed the highest reproducibility. The variation in reproducibility can be ascribed to the increase in sample handling during processing especially in relation to recovery of insoluble material, namely holocellulose (from which lignin yields were calculated) and α -cellulose. Lignin yields generally exceeded the published values of 18-24%. Yields of α -cellulose and hemicellulose showed the greatest consistency with the established values, these being 29.9-32.4% and 23-32% respectively.

Extractives

Consistent yields (**Table 1**) were obtained for 24 h Soxhlet extractions of un-ground trial samples (TSR1-3; 16.3-22.4 %) and the ground trial sample (TSR11; 16.4%). Extraction for 12 h yielded a lower level of extractives (TSR4-6; 13.6-15.7%), while extraction for 8 hr yielded extractive levels closer to the lower end of this range (TSR7-8; 13.4-13.6%). Consistent yields of extractives were also obtained for varieties WI 81456 (15.5%) and DB 7869 (15.2%), which were subjected to 12 h extractions. These yields were lower than those obtained for the 24 hr extraction period. It is to be noted however, that the method of sample preparation prior to extraction may have influenced the level of extractives; knifed cane from the factory has a higher level of inorganic components and cane trash than the chopped pressed cane.

Lignin

Delignification for 5 h following the 24 h Soxhlet extraction resulted in the lowest yield of lignin (6.18-10.2%) from the trial sample (**Table 1**; TSR1-3). The yields recorded were below the published range of 18-24%. An increase in the lignin yield to 20.3%, which is within the expected range was obtained with an increase in delignification time to 24 h following a 12 h extraction (TSR4). This is indicative of delignification not being completed during the 5 h delignification period used initially. Lignin yields slightly higher than the higher limit were obtained using a delignification time of 10 h following 12 h of Soxhlet extraction (TSR9; 27.4%). Twenty-four (24) hours of delignification following 8 h Soxhlet extraction (TSR7-8) yielded on average 35% lignin, which was the highest yield recorded in the study. Lignin yields for other delignification/extraction regimes applied to both the composite samples (TSRs) and the distinct varieties WI 81456 and DB 7869, ranged from 29.1-32.9%.

The high lignin values generally recorded for both the composite trial sample (TSRs) and the varieties WI 81456 and DB7869 compared with the published values seem to suggest that there was inefficient separation of the insoluble holocellulose from the soluble lignin following delignification. This step that was effected by vacuum filtration was considered the most difficult step in the Jayme-Wise procedure. A considerable effort had to be made to recover “fines” from the filtrate. It is possible that some “fines” that could not be recovered using the method employed were lost to the soluble lignin fraction thereby contributing to an enhancement of the lignin levels. Consideration therefore needs to be given to enclosing the holocellulose in a bag made out of a suitable material or by using sintered glass crucibles or some other type filter paper in an attempt to effect

better separation. The extraction time also appeared to be a factor, given the higher lignin yield obtained from samples subjected to 8 hr of Soxhlet extraction. The possibility that the samples analysed have an inherently higher level of lignin than the levels reported also warrants some consideration.

α -Cellulose and Hemicellulose

Alkaline hydrolysis of the holocellulose for 24 h at 4 °C and 1.25 h at 26 °C gave comparable yields of α -cellulose and hemicellulose. The above normal levels of α -cellulose in TSR2 and TSR3, and hemicellulose in TSR1 are viewed as distortions caused by the low lignin recovery. The lower α -cellulose levels obtained for several of the analyses (**Table 1**) compared to the published values of 29.9-32.4% for bagasse can be attributed to the low holocellulose recovery due to enhancement of the lignin recovery.. Given the fact that the majority of the hemicellulose levels detected were within the published range of 23-32% for bagasse, the loss of holocellulose would appear to have had a more notable impact on α -cellulose yields compared to hemicellulose yields.

CONCLUSION

This study has demonstrated that a minimum Soxhlet extraction time of 12 h is required for removal of extractives. The study also clearly showed that delignification periods of 15-24 h as opposed to 5 h favoured improved lignin yields, and that alkaline hydrolysis for a period of 1.25 h at 26 °C generally provided yields of α -cellulose and hemicellulose comparable to the 24 h treatment at 4 °C. The former alkaline hydrolysis regime is preferable because of the reduced reaction time. The inability to obtain yields of lignin within the established limit of 18-24%, in spite of the development of a more effective delignification regime is attributable to inefficient separation of holocellulose. To this end an examination of alternative sample handling and separation procedures for holocellulose, and to a lesser extent hemicellulose is critical to the continuation of this study. Use of certified reference materials or some other reference material and/or comparisons of results using internationally accepted reference methods of analysis of lignin, α -cellulose and hemicellulose would also be useful for quality assurance. The relative composition of the fibre in the contrasting varieties of sugarcane examined in this study, were of the same order of magnitude. This procedure should have the potential to contribute to the assessment of the potential of sugarcane varieties for fuel or other utilisation of fibre.

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Table 1. Levels (mean \pm SE) and of α -cellulose, hemicellulose, lignin and extractives in sugar-cane fibre (bagasse) using different extraction, delignification and alkaline hydrolysis regimes.

Sample	Pre-treatment Regime	Extraction/ Delignification Time (hr)	Alkaline Hydrolysis Regime Time (hr) & Temperature ($^{\circ}$ C)	α -Cellulose (%) [29.2-32.4] ¹	Hemicellulose (%) [23-32] ¹	Lignin (%) [18-24] ¹	Extractives (%)
TSR1 ²	Unground	24/5	24 @ 4	29.3 \pm 3.00	46.3 \pm 3.00	6.18 \pm 0.38	18.4 \pm 0.40
TSR2	Unground	24/5	24 @ 4	36.6 ³	30.5	10.2	22.4
TSR3	Unground	24/5	24 @ 4	45.6 \pm 0.70	31.7 \pm 1.20	6.40 \pm 0.62	16.3 \pm 0.10
TSR4	Unground	12/24	1.25 @ 26	34.6 \pm 0.15	30.7 \pm 0.10	20.3 \pm 0.10	14.5 \pm 0.10
TSR5	Ground	12/24	1.25 @ 26	25.4 ³	27.9	30.8	15.7
TSR6	Ground	12/24	1.25 @ 26	27.2 \pm 0.20	26.1 \pm 0.10	32.9 \pm 2.30	13.6 \pm 0.65
TSR7	Ground	8/24	1.25 @ 26	25.8 \pm 0.60	25.6 \pm 0.75	35.3 \pm 0.05	13.4 \pm 0.05
TSR8	Ground	8/24	1.25 @ 26	29.6 \pm 1.60	21.8 \pm 0.70	35.2 \pm 1.35	13.6 \pm 0.45
TSR9	Ground	12/10	1.25 @ 26	26.7 \pm 0.00	30.3 \pm 1.15	27.4 \pm 1.35	15.8 \pm 0.15
TSR10	Ground	12/15	1.25 @ 26	31.3 \pm 1.05	24.2 \pm 0.30	29.1 \pm 1.55	15.5 \pm 0.20
TSR11	Ground	24/15	1.25 @ 26	31.0 \pm 0.40	20.0 \pm 0.75	32.7 \pm 0.35	16.4 \pm 0.05
WI 81456 – Fuel Cane	Ground	12/24	1.25 @ 26	31.6 \pm 0.25	22.4 \pm 0.35	30.8 \pm 0.05	15.5 \pm 0.00
DB 7869 – Industry Standard	Ground	12/24	1.25 @ 26	31.3 \pm 0.55	24.3 \pm 0.15	29.3 \pm 0.50	15.2 \pm 0.10

1. Literature Values

2. Trial sample composite of several commercial varieties;

3. Loss of sample occurred during processing of duplicate.