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T 249

DRAFT NO. 03 SARG

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WORKING GROUP
CHAIRMAN Bogdan Marian Tofanica

SUBJECT
CATEGORY Chemical Properties

RELATED
METHODS See "Additional Information"

CAUTION:
This method may require the use, disposal, or both, of chemicals which may present serious health hazards to humans. Procedures for the handling of such substances are set forth on Safety Data Sheets which must be developed by all manufacturers and importers of potentially hazardous chemicals and maintained by all distributors of potentially hazardous chemicals. Prior to the use of this test method, the user should determine whether any of the chemicals to be used or disposed of are potentially hazardous and, if so, must follow strictly the procedures specified by both the manufacturer, as well as local, state, and federal authorities for safe use and disposal of these chemicals.

Carbohydrate composition of extractive-free wood and wood pulp by gas-liquid chromatography

(Ballot for reconfirmation T 249 cm-09 as a Classical Method)

(No changes from draft 1, proceed to SARG)

1. Scope

This method (*1*, *2*) is used to determine the five principal monosaccharides which define the carbohydrate composition of wood and wood pulp. The constituents determined quantitatively and on an absolute basis are glucan, mannan, arabinan, xylan and galactan. Concentrations of the individual components as low as 0.1% can be determined. The method is applicable to extractive-free wood as well as to wood pulp.

2. Summary

Samples are hydrolyzed with sulfuric acid using a two-step technique. A portion of the hydrolyzate is

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neutralized, and the sugars are reduced to the alditols with sodium borohydride. The alditols are acetylated with acetic anhydride and sulfuric acid, and the alditol acetates are precipitated in ice water and extracted with methylene chloride for injection into the gas chromatograph.

3. Significance

3.1 The carbohydrate composition of a pulp is important in determining its response to processing conditions and the development of physical properties. In addition, the various carbohydrate constituents in wood have different sensitivities to pulping chemicals, and this affects pulp yield and quality. While relative ratios of the monosaccharide constituents may be satisfactory for characterization of dissolving pulps, it is desirable to obtain absolute values for paper grade pulps due to their more complex composition.

3.2 The reaction conditions employed in the hydrolysis and derivatization procedures have been selected to minimize losses of the monosaccharides; however, it is known that the losses do occur. These are accounted for by subjecting the monosaccharides to these reaction conditions in the calibration step and by use of an internal standard.

4. Apparatus

4.1 *Gas chromatograph*, single or dual column instrument with flame ionization detector and equipment for measuring peak areas, e.g., disc chart integrator or electronic integrator. The alditol acetates can be chromatographed satisfactorily on a 316 stainless steel or glass 1.83 m × 3 mm (6 ft × 1/8 in.) column packed with 3% ECNSS-M on Gas Chrom Q 100/120 mesh (Applied Science Laboratories). The column can be operated isothermally at 190°C with an injection port temperature of 200°C and a detector temperature of 250°C. Helium carrier gas can be used with a flow rate of 30 mL/min. With some instruments it may be necessary to modify the injector port to reduce dead space and allow on-column injection to avoid "ghosting." (In light of developments in capillary column technology, alternative chromatography columns, operating conditions and temperature programming may be used in place of those specified in this method.)

NOTE 1: For convenience, the column temperature can be adjusted to obtain base line resolution of the components and elution of inositol in 30-45 min. Conditioning the column at 220°C, which is above its maximum temperature, will reduce retention times. As the column ages, the temperature required for satisfactory resolution decreases. Satisfactory results have been obtained with column temperatures as high as 205°C and the injection port at 210°C.

4.2 *Flash evaporator*, rotary, thin film, vacuum type with water bath.

4.3 *Drying oven*, constant temperature, with means of ensuring adequate temperature control (105 ± 3°C) and free access of air.

4.4 *Constant temperature water bath*, regulated to 30 ± 0.5°C.

4.5 *Water bath*, regulated to 50-60°C.

4.6 *Autoclave*, capable of operation at 103 ± 7 kPa (15 ± 1 psi).

4.7 *Other apparatus*: conical glass centrifuge tubes, 15-mL; glass stirring rods drawn to a point; beakers, 50 and 250 mL; graduated cylinders, 10 and 100 mL; round bottom flasks, 100-mL, to fit flash evaporator; pipet, 10-mL; tapered centrifuge tubes, 50-mL, plastic or glass; separatory funnels, 250-mL; magnetic stirrer and stirring bars. Automatic pipets are useful for dispensing sulfuric acid and acetic anhydride.

5. Reagents

5.1 *Monosaccharides*, for calibration: B-D(+) glucose, D(+) mannose, L-arabinose, D(+) galactose, and D(+)xylose.

5.2 *Myo-inositol*, reagent grade, for internal standard. Prepare a solution containing 1.000 g of myo-inositol in 100.0 mL of distilled water. Check the chromatographic purity as described in Appendix B to ensure that the inositol does not contain any components which would interfere with the monosaccharides to be measured. Each time a new batch of inositol is introduced, the calibration constants for the individual monosaccharides must be reestablished.

5.3 *Sulfuric acid*, 72% H_2SO_4. To 300 mL of water in a 1000-mL volumetric flask, add slowly while cooling under a cold water tap, 670 mL of concentrated sulfuric acid (H_2SO_4 sp gr 1.84). When temperature has equilibrated to ambient, dilute to the mark and mix. Standardize against 0.5*N* sodium hydroxide. Adjust the acid strength to 72 ± 0.1% by weight.

5.4 *Barium hydroxide*. Prepare a saturated aqueous solution of $Ba(OH)_2$.

5.5 *Bromophenol blue indicator*. Prepare a 0.1% aqueous solution.

5.6 *Other reagents*: sulfuric acid, concentrated; sodium borohydride; acetic acid, glacial; acetic anhydride; methanol, anhydrous; methylene chloride.

6. Sampling

6.1 *Pulp samples*. Obtain a sample of pulp weighing approximately 2.5 g, according to a predetermined sampling procedure, and grind the sample to pass a 40-mesh screen.

6.2 *Wood samples*. Obtain a sample of wood weighing more than 5 g, according to a predetermined sampling procedure.

6.2.1 Prepare approximately 5 g of extractive-free wood according to TAPPI T 264 "Preparation of Wood for Chemical Analysis" using the total sample which passes through the 40-mesh screen.

7. Test specimens

Allow the sample to come to moisture equilibrium in the atmosphere near the balance, and weigh two test specimens of 0.35 ± 0.01 g, to the nearest 0.1 mg, into 15-mL conical glass centrifuge tubes. At the same time weigh another 2.0-g specimen for moisture determination as described in TAPPI T 412 "Moisture in Paper and Paperboard."

8. Procedure

8.1 The neutralized hydrolyzate may not be stable; however, the alditols are quite stable. Therefore, the hydrolysis (8.2), neutralization and concentration (8.3), and reduction (8.4) steps below must be completed with out interruption. This can be done on a set of 4-6 specimens in a single day.

8.2 *Hydrolysis*

8.2.1 To the specimen in the centrifuge tube add exactly 3 mL of 72% sulfuric acid with a pipet. Stir the contents of the tube with a tapered stirring rod until the specimen begins to dissolve.

8.2.2 Place the tube in a 30 ± 0.5°C water bath for one hour. Stir occasionally while being sure to keep the tube in the bath.

8.2.3 Wash the contents of the centrifuge tube into a 250-mL beaker with 84 mL of water. It is very important at this stage of the procedure to be sure that the entire specimen solution is transferred from the centrifuge tube to the beaker. Great care must be taken to avoid any losses. If *any* specimen solution is lost during the hydrolysis step, terminate the analysis, weigh out a new specimen, and proceed again.

8.2.4 Cover the beaker with a watch glass, and place in an autoclave at 103 ± 7 kPa (15 ± 1 psi) for 1 h.

8.3 *Neutralization and concentration*

8.3.1 Cool the specimen solution to room temperature using an ice bath.

8.3.2 Pipet 10 mL of the internal standard solution into the cooled solution.

8.3.3 After mixing, transfer 10 mL of the solution to a 50-mL beaker.

8.3.4 Add several drops of bromophenol blue indicator.

8.3.5 Add a magnetic stirring bar, and while stirring on a magnetic stirrer add saturated barium hydroxide solution until the color of the solution changes from yellow to blue-violet.

NOTE 2: When adding the barium hydroxide, exercise care so that the amount added does not exceed that required for the indicator color change. If the solution becomes alkaline, reversion of the monosaccharides may occur. The sensitivity to reversion may be reduced by finishing the neutralization with 0.04*N* barium hydroxide or by neutralizing the entire hydrolyzate with 0.4*N* barium hydroxide, centrifuging and using about 25 mL of clear supernatant for the reduction.

8.3.6 Transfer the contents of the beaker to a 50-mL tapered centrifuge tube and centrifuge until the supernatant liquid is clear.

8.3.7 Decant most of the clear solution into a 100-mL round bottom flask.

8.4 *Reduction*

8.4.1 Add 80 mg of sodium borohydride, mix, and swirl occasionally for 1.5-2.0 h at room temperature.

8.4.2 Destroy the excess borohydride by adding glacial acetic acid dropwise until gas evolution ceases.

8.4.3 Concentrate the solution to a syrup on a flash evaporator with the water bath set at ≤ 40°C.

8.4.4 Add about 10 mL of methanol to the syrup, mix, and evaporate to dryness.

8.4.5 Repeat step 8.4.4.

8.4.6 Heat the residue in the flask in an oven at 105°C for 15 min to ensure complete removal of the water.

8.5 *Acetylation*

8.5.1 To the dry residue from step 8.4.5, add 7.5 mL of acetic anhydride and 0.5 mL of sulfuric acid.

8.5.2 Place the flask in a water bath maintained at 50-60°C for 1 h.

8.5.3 Remove the flask from the water bath and allow it to cool for about 5 min.

8.5.4 Pour the contents of the flask slowly with stirring into about 70 mL of a water-ice mixture (ca 30 water and 40 g ice) contained in a 250-mL beaker.

8.5.5 Transfer the mixture to a 250-mL separatory funnel and extract successively with 25-, 15-, and 10-mL portions of methylene chloride (lower layer) and combine the methylene chloride extracts in a 100-mL round bottom flask.

8.5.6 Using the flash evaporator with the water bath at 75°C, concentrate the combined methylene chloride extracts to near dryness.

8.5.7 Add about 1 mL of water to the residue and evaporate to dryness on the flash evaporator.

8.5.8 Dissolve the residue in 2 mL of methylene chloride.

8.6 *Chromatography*. Inject about 0.5 µL of the solution of alditol acetates from step 8.5.8 into the gas chromatograph and determine the peak areas for each of the five monosaccharides and the internal standard.

9. Calculation

The percent of each component as polysaccharide is calculated according to the following equation:

$$\text{Percent} = \frac{A \times W_s \times C \times 100}{A_s \times W \times k}$$

where:

A = chromatographic area of component peak

A_s = chromatographic area of internal standard peak

W_s = weight of internal standard, mg (100 mg)

W = oven dry weight of sample, mg

C = conversion factor for monosaccaride to polysaccaride (0.88 for pentoses, 0.90 for hexoses)

k = calibration factor for each individual component

10. Calibration

10.1 Monosaccharide standards in the amounts applicable to pulp samples are run through the entire procedure to establish the gas chromatographic response factors and to account for the hydrolysis survival for each component. A set of typical calibration samples is given in Table 1. The sugars are weighed most conveniently into 15-mL centrifuge tubes using a microbalance. If a microbalance is not available, the weights may be increased by a factor of 10 and the hydrolysis scaled up to a level of 34.5 mL of 72% sulfuric acid in step 8.2.1. Then in step 8.2.3, transfer the solution to a 1000-mL volumetric flask and dilute to the mark with water. Take a 100-mL aliquot of this solution and proceed with step 8.2.4.

Table 1. Composition of calibration mixtures

	Mixture number					
Sugar	*1*	*2*	*3*	*4*	*5*	*6*
Glucose, mg	270.0	255.0	240.0	225.0	215.0	180.0
Xylose, mg	17.5	22.0	26.0	32.0	36.0	55.0
Mannose, mg	9.0	12.5	23.0	27.5	32.0	46.0
Arabinose, mg	1.7	3.2	6.4	9.0	1.5	20.0
Galactose, mg	1.0	1.5	2.8	3.5	4.5	5.5
Total sugar, mg	229.2	294.2	298.2	297.0	299.0	306.5

10.2 Run the calibration mixtures through steps 8.2 and 8.6.

10.3 Calculate the calibration constant, k, for each monosaccharide as follows:

$$k = \frac{A_c \times W_s}{A_s \times W_c}$$

where:

A_c = chromatographic area of component peak
W_s = weight of internal standard, mg (100 mg)
A_s = chromatographic area of internal standard peak
W_c = weight of component, mg

10.4 If there is any doubt about the chemical or chromatographic purity of the standards, they should be assayed as described in Appendixes A and B, and corrections should be made to the weights of the individual constituents as required.

11. Report

Report the average of the duplicate determinations for each component, to the nearest 0.1%, based on the oven-dry specimen weight.

12. Precision

12.1 The following estimates of precision are based on a study of 15 southern pine pulps removed from the kraft process at various points throughout the cooking schedule and analyzed in two laboratories.

12.2 Repeatability (within a laboratory) and reproducibility (between laboratories):

Component	*Repeatability, %*	*Reproducibility, %*
Glucan	2. 3	3.0
Mannan	0. 47	1.6
Xylan	0.47	0.75
Arabinan	0. 31	0.89
Galactan	0. 47	0.36
Total carbohydrates	2.5	4.6

12.3 A long-term repeatability study covering a period of 5 months (25 determinations) was conducted at one laboratory using a single kraft pulp with the following results:

Component	*Average, %*	*Reproducibility, %*
Glucan	63.3	3.8
Mannan	7.3	0.64
Xylan	7.6	0.58
Arabinan	1.3	0.15
Galactan	1.6	0.89
Total carbohydrates	81.1	4.4

12.4 These values are given in accordance with the definitions of these terms in TAPPI T 1206 "Precision Statement for Test Methods."

13. Keywords

Wood, Pulp, Carbohydrates, Monosaccharides, Glucan, Mannan, Arabinan, Xylan, Galactan, Gas chromatography

14. Additional information

14.1 Effective date of issue: To be assigned.

14.2 Related method: ASTM D 1915.

14.3 This method, formerly T 249 pm-75, has been reclassified as a Classical Method. Such procedures are no longer in common use or have been superseded by advanced technology; they are technically sound, have a history of use, and contain a body of literature references that make their preservation valuable.

Appendix A. Chemical analysis of standards

A.1 *Scope and summary.* This procedure is used to determine the chemical purity of monosaccharides used as calibration standards for determination of carbohydrate composition of wood and pulps. The sugars are oxidized with excess periodic acid which is specific for the 1, 2 glycol structure. The excess periodic acid is determined volumetrically using the arsenite-iodine system.

A.2 *Apparatus*: beakers, 50 and 1000 mL; volumetric flasks, 500 and 1000 mL; Erlenmeyer flasks, 250 and 500 mL; pipets, 50 mL; graduated cylinders, 10 and 100 mL; buret, 50 mL.

A.3 *Reagents*

A.3.1 *Periodic acid* (0.1*N*). Weigh 11.501 g of potassium metaperiodate (KIO_4) into a 1000-mL beaker. Add 500 mL of water and 100 mL of 1*N* H_2SO_4. Stir and heat until dissolved and transfer solution to a 1000-mL volumetric flask. Cool and dilute to volume.

A.3.2 *Sodium arsenite* (0.12*N*). Accurately weigh 5.9346 g of arsenic trioxide (primary standard grade) into a 1000-mL volumetric flask, add 120 mL of 1*N* NaOH and heat to dissolve. Add 100 mL of H_2O, 2 drops of phenolphthalein and acidify with 3*N* HCl, then add 2 drops in excess. Cool and dilute to volume.

A.3.3 *Iodine solution* (0.12*N*). Dissolve 40 g of potassium iodide in 25 mL of H_2O contained in a 50-mL beaker. To this solution add 15.3 g of iodine. Mix to dissolve. Transfer the solution to a 1000-mL volumetric flask and dilute to volume with water.

A.3.4 *Starch indicator.* Prepare a 5% solution of starch of formamide by making a slurry with a small amount of formamide and adding it, while stirring, to the remainder of the formamide at 100-110°C.

A.3.5 *Saturated sodium bicarbonate solution*, $NaHCO_3$.

A.3.6 *Potassium iodide*, 10% solution KI.

A.4 *Standardization of iodine solution.*

A.4.1 Pipet 25 mL of the arsenite solution (A.3.2) into a 250-mL Erlenmeyer flask and add 25 mL of water.

A.4.2 Add 80 mL of bicarbonate solution and 4 mL of 10% potassium iodide solution.

A.4.3 Calculate the normality of the iodine solution using the following equation:

$$N = \frac{25 \times W}{V \times E}$$

where:

W = weight of arsenic trioxide
V = volume of iodine solution
E = equivalent weight of arsenic trioxide, 49.45

A.5 *Analysis of sugars*

A.5.1 Weigh accurately a 600-mg specimen into a 500 mL volumetric flask. Dissolve and dilute to volume with water.

A.5.2 Transfer a 50 mL aliquot of the solution to a 500-mL flask.

A.5.3 Prepare a blank using just 50 mL of water in a 500-mL flask.

A.5.4 Pipet exactly 50 mL of the periodic acid into each flask. Mix and allow to stand for 1 h.

A.5.5 Add 80 mL of bicarbonate solution followed by exactly 50 mL (pipet) of arsenite solution and 4 mL of potassium iodide solution.

A.5.6 Add about 1 mL of starch indicator and titrate with iodine solution to an indicator color change from colorless to blue.

A.5.7 Calculate the percent sugar in the specimen according to the following equation:

$$\% = \frac{(V - V_b) \times N \times \mathrm{MW} \times 100}{2 \times E \times W}$$

where:

V = volume titrant for specimen, mL
V_b = volume titrant for blank, mL
N = normality of iodine solution
W = weight of specimen, mg
MW = molecular weight of sugar analyzed
E = number of adjacent hydroxyl groups

Sugar	*MW*	*E*
Xylose	150.13	4
Arabinose	150.13	4
Galactose	180.16	5
Mannose	180.16	5
Glucose	180.16	5

Appendix B. Gas chromatographic purity of standards

B.1 The gas chromatographic purity of the sugar standards used to calibrate this method is determined by weighing 500 mg of the respective sugar into a 100-mL round-bottom flask. The specimen is then dissolved in 10 mL of water and treated as a normal specimen from Step 8.4 in the procedure.

B.2 The proportion of the traces of other sugars in the sample analyzed is determined on an area ratio basis using the following equation:

$$\text{Purity factor} = \frac{A_c}{A}$$

where:

A_c = area of component peak

A = sum of area of component peaks

The purity factor is calculated for each of the five sugars present in each standard.

References

1. Crowell, E.P. and Burnett, B.B., *Analytical Chemistry* **39**:121 (1967).
2. Borchardt, L.G. and Piper, C.V., "A Gas Chromotographic Method for Carbohydrates as Alditol-Acetates," *Tappi* **53**(2):257 (1970).

Your comments and suggestions on this procedure are earnestly requested and should be sent to the TAPPI Standards Department. ■