INTERNATIONAL STANDARD

ISO 21437

First edition
2020-12

Pulps — Determination of carbohydrate composition

Pâtes — Détermination de la composition des hydrates de carbone

Reference number
ISO 21437:2020(E)

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Published in Switzerland

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 6, *Paper, board and pulps*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

The carbohydrate composition - the contents of the five principal, neutral monosaccharides; arabinose, galactose, glucose, xylose and mannose - provides chemical information about the main polysaccharides in wood pulps. The most commonly-used methods are based on acid hydrolysis of the polysaccharides using sulfuric acid, followed by subsequent chromatographic determination of the monosaccharides.

This document describes a method for the determination of the contents of the five principal, neutral monosaccharides; arabinose, galactose, glucose, xylose and mannose, as they appear in wood pulps. The procedure is based on the sulfuric acid hydrolysis of the samples. The monosaccharides are determined either by using high performance anion exchange chromatography with a pulsed amperometric detector (HPAEC-PAD) – subsequently referred to as ion chromatography (IC), or by using gas chromatography with a flame ionization detector (GC-FID) – subsequently referred to as gas chromatography (GC).

The determination of carbohydrate composition can also be carried out by HPLC (High Performance Liquid Chromatography), as described, for example, in Reference [3], provided that the results have been validated against those obtained with this document.

Pulps — Determination of carbohydrate composition

1 Scope

This method describes the determination of the carbohydrate composition in wood pulp samples. This method makes it possible to determine concentrations of individual anhydrous monosaccharides down to 1 mg/g oven-dry pulp.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 638, *Paper, board and pulps — Determination of dry matter content — Oven-drying method*

ISO 7213, *Pulps — Sampling for testing*

ISO 14453, *Pulps — Determination of acetone-soluble matter*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
carbohydrate composition
amounts of the five principal, neutral monosaccharides; arabinose, galactose, glucose, mannose and xylose, in a sample, in milligrams per gram

4 Principle

The pulp samples are hydrolysed with sulfuric acid using a two-step technique. The amounts of the different monosaccharides are determined using either ion chromatography (IC) or gas chromatography (GC) in the presence of an internal standard to validate the results. If GC is used, the hydrolysed sample is reduced and acetylated, and the resulting alditol acetates of the monosaccharides are then separated and determined by GC.

5 Apparatus

5.1 Grinder with a 40 mesh screen or equivalent equipment.

5.2 Filtration equipment: filtering flask; filtering crucible, fritted glass, medium or fine porosity, 30 ml; adapter for the filtering crucible, siphon tube (optional).

NOTE The choice of fritted glass porosity depends on the rate of filtration of the particular type of sample. For slow filtering samples, the use of medium (M) porosity is preferable. In low-yield sulfite pulps especially, lignin forms a fine dispersion and clogs the pores of the filter. Filtration can be facilitated by using a medium porosity crucible with a disc of fine porosity glass-fibre filter paper fitted over the sintered glass in the crucible.

Other types of filtering crucibles, such as alundum or porous porcelain crucibles lined with a mat of glass fibres can also be used.

5.3 Constant temperature water bath, capable of maintaining a temperature of 30 ± 1 °C.

5.4 Autoclave, capable of maintaining a temperature of 120 ± 3 °C.

5.5 Drying oven, 105 ± 2 °C for determining dry matter content in accordance with ISO 638.

The apparatus in 5.6 is used for IC determination only:

5.6 Ion chromatograph (IC) with an anion-exchange column for monosaccharide determination and pulsed amperometric detector (PAD).

Apparatuses in 5.7 and 5.8 are used for GC-determinations only:

5.7 Water bath, maintained at a temperature of 40 ± 0,5 °C.

5.8 Gas chromatograph (GC) with a suitable column for monosaccharide determination and flame ionization detector (FID).

6 Reagents

All chemicals shall be of reagent grade or better, unless otherwise indicated.

6.1 Water of high purity, distilled or deionized

6.2 Monosaccharide standards

Monosaccharide standards, for calibration: arabinose, galactose, glucose, mannose and xylose. Prepare standard solutions of appropriate concentrations, each standard solution containing all five monosaccharides.

6.3 Sulfuric acid, 72 % w/w (specific gravity 1,633 8 at 20 °C). 72 % sulfuric acid can be prepared from concentrated sulfuric acid as follows:

Slowly add 650 ml of concentrated sulfuric acid (H_2SO_4 sp gr 1,84) to 400 ml of water, while cooling under a cold-water tap. When the temperature has reached equilibrium with the ambient temperature, adjust the specific gravity of the acid solution to 1,633 8 with the use of a hydrometer, by careful addition of concentrated sulfuric acid or water.

NOTE 72 % H_2SO_4 is also available commercially.

6.4 Eluent solution (for IC determination)

The composition of this solution depends on the type of IC column to be used. Therefore, follow the recommendations given by the IC column supplier.

Reagents 6.5 to 6.12 are required only for GC determinations.

NOTE Alternative reagents (to those in 6.5 to 6.12) and procedures (to those in 9.4.2.1, 9.4.2.2, and 9.4.2.3) for the neutralization, reduction and derivatization steps, such as those described in TAPPI T249[2], can also be used provided this is indicated in the report.

6.5 Ammonia, NH_3 conc. 25 %

6.5.1 Ammonia 13 M

Mix 9 parts ammonia (25 %) with 1 part of water.

6.6 Potassium hydroxide, KOH

6.6.1 Potassium hydroxide 7,5 M

Weigh 105 g KOH pellets into a 250 ml beaker. Add approx. 150 ml water while stirring. Transfer the solution into a 250 ml volumetric flask, using an additional 20-30 ml of water to rinse the beaker in order to complete the transfer.

The reaction is exothermic; heat is evolved. Allow the solution to cool to ambient temperature and dilute to the mark with water.

6.7 Potassium borohydride, KBH_4

6.7.1 Potassium borohydride solution

Dissolve 150 mg KBH_4 in 250 µl 13 M NH_3 and 750 µl distilled water in a septum vial (4 ml).

This solution shall be freshly prepared every day before use.

6.8 Acetic acid, CH_3COOH, conc.

6.9 Acetic acid anhydride, conc.

6.10 1-methylimidazole

6.11 Ethanol, 95 - 99 %

6.12 Sodium sulfate, Na_2SO_4, water-free

6.13 2-Deoxy-galactose

6.13.1 Internal standard solution, 2-deoxy-galactose 20 mg/ml

Weigh 1,00 g 2-deoxy-galactose to the nearest 0,1 mg, transfer it quantitatively to a 50 ml volumetric flask, and dilute to the mark with distilled water.

Other internal standards than 2-deoxy-galactose, such as fucose or myo-inositol, can also be used. However, this shall be specified in the report.

7 Sampling

If the analysis is being made to evaluate a lot of a consignment of pulp, the sample shall be taken in accordance with ISO 7213. If the analysis is made on another type of sample, report the origin of the sample, and if possible, the sampling procedure. Pulp samples shall be ground before analysis using an

appropriate grinder (5.1). Groundwood and high yield pulps containing a significant amount of resins shall be extracted with acetone according to ISO 14453 before testing.

NOTE Resins, if not extracted from the pulp prior to analysis, would remain insoluble in acid and can interfere with the determination of carbohydrate composition.

NOTE Acetone is considered an effective solvent for extracting resin from pulp. Dichloromethane and ethanol/benzene (1:2), as specified in other methods, are not recommended due to health hazards. In particular, benzene is a confirmed carcinogen.

8 Test Specimens

Make sure that the test portions taken are representative of the sample received.

9 Procedure

Carry out the preparation and testing in duplicate.

9.1 Determination of dry matter content

Determine the dry matter content of the pulp according to ISO 638 by drying a 2-3 g specimen in an oven (5.5) at 105 °C ± 2 °C to constant weight. If the pulp needs to be pre-extracted, the dry matter content shall be determined on the extracted pulp.

9.2 Test specimen preparation for acid hydrolysis

Weigh a portion, of the sample from Clause 7, of 300 ± 10 mg to the nearest 0,1 mg into a glass beaker with a volume of at least 150 ml.

Calculate and record the oven-dry mass *M* of the test specimen, in grams.

9.3 Hydrolysis

To the test specimen in the beaker, add exactly 3 ml of 72 % sulfuric acid (6.3) with a pipette. Stir the contents of the beaker with a glass rod until the test material begins to dissolve. Leave the glass rod in the beaker.

NOTE Some pulps do not absorb the acid and therefore do not disperse readily. In such cases, after addition of acid, place the beaker under vacuum in a vacuum desiccator for at least 15 min to facilitate wetting and absorption.

Place the beaker in a 30 ± 1 °C water bath (5.3) for 1 h. Stir occasionally with the glass rod. Add 84 ml of water (6.1), using some of this water to rinse the glass rod, making sure all the washing is captured in the beaker.

Cover the beaker with aluminium foil and place it in autoclave (5.4) at 120 ± 3 °C for 1 h. Allow the beaker and its contents to cool to approximately 80 °C.

9.4 Determination of monosaccharides

Carry out the determination according to either 9.4.1 or 9.4.2.

NOTE The determination of carbohydrate composition can also be carried out by HPLC (High Performance Liquid Chromatography), as described in Reference [3] for example, provided that the results have been validated against those obtained with this document.

9.4.1 Determination using an IC instrument

9.4.1.1 Solution preparation

Without stirring the residue remaining after hydrolysis (from 9.3), which consists primarily of insoluble lignin, decant or siphon off the supernatant solution through a filtering crucible (5.2) into a 250 ml filtering flask (5.2). Transfer the filtrate to a 250 ml volumetric flask. Wash the precipitate with 2 × 30 ml warm water (6.1) and add the washings to the volumetric flask. Add an appropriate volume of the internal standard 2-deoxy-galactose solution (6.13.1). Allow it to cool to room temperature and fill up to the mark with water.

NOTE 1 The acid-insoluble lignin and acid-soluble lignin can be determined from the residue after hydrolysis, and from the filtrate in the volumetric flask, respectively, as described in ISO 21436.

NOTE 2 Transfer of the filtrate to a volumetric flask is not necessary if the determination of acid-soluble lignin is not required.

NOTE 3 Addition of the internal standard at this stage ensures that the ratio of monosaccharides to internal standard remains fixed throughout the entire procedure.

9.4.1.2 Calibration

Calibrate the device using the monosaccharide standard solutions (6.2) containing the internal standard 2-deoxy-galactose (6.13.1). Use the conditions recommended by the manufacturer or determine the optimum conditions empirically. The optimum conditions depend on the apparatus and the column.

9.4.1.3 Determination of chromatographic areas of monosaccharides

Filter the test solution, from Clause 9.4.1.1, through a syringe filter, with a PVDF membrane with a pore size of 0,45 µm or equivalent. Dilute this sample to a concentration that is within the calibration range. Inject an aliquot into the instrument.

Run the determination according to the manufacturer's instructions.

Check from the chromatogram that the peak separation is adequate. If necessary, dilute the sample further until the concentration is within the calibration range. Run a new determination.

Determine the chromatographic area A_i for each monosaccharide.

9.4.2 Determination using a GC instrument

Without stirring the residue remaining after hydrolysis (from 9.3), consisting primarily of insoluble lignin, decant the supernatant solution through a filtering crucible (5.2) into a 100 ml filtering flask (5.2). Transfer the filtrate to a 100 ml volumetric flask and add 2 ml of the standard deoxy-galactose solution (6.13.1).

Wash the precipitate with 2 × 5 ml hot distilled water and add the washings to the volumetric flask. Allow the sample to cool and dilute with distilled water to the mark.

NOTE 1 The acid-insoluble lignin and acid-soluble lignin can be determined from the residue after hydrolysis, and from the filtrate in the volumetric flask, respectively, as described in ISO 21436.

NOTE 2 Transfer of the filtrate to a volumetric flask is not necessary if the determination of acid-soluble lignin is not required.

NOTE 3 Addition of the internal standard at this stage ensures that the ratio of monosaccharides to internal standard remains fixed throughout the entire procedure.

NOTE 4 Alternative reagents (to those in 6.5 to 6.12) and procedures (to those in 9.4.2.1, 9.4.2.2, and 9.4.2.3) for the neutralisation, reduction and derivatization steps, such as those described in TAPPI T249[2], can also be used provided this is indicated in the report.

9.4.2.1 Neutralisation

Transfer 1 ml of the solution (from 9.4.2) to a test tube.

Add 100 µl of 13 M ammonia (6.5.1). Check with a glass capillary and a pH indicator paper that the pH is >7.

9.4.2.2 Reduction

Add 100 µl of freshly prepared KBH_4-solution (6.7.1) to the neutralized supernatant solution from 9.4.2.1 and place the test tube in a water bath (5.7) at 40 °C for 1 h.

Add 100 µl of conc. acetic acid (6.8) to eliminate the excess of KBH_4.

9.4.2.3 Derivatisation

Transfer 500 µl of the sample to a 30 ml test tube with a screw cap. Add 500 µl of 1-methylimidazole (6.10) and 5 ml of acetic acid anhydride (6.9). Cool the tube with cold water during the addition of the acetic acid anhydride.

Tighten the cap and mix the solution carefully. Allow the tube to stand in a cold-water bath for 10 min. Add 1,0 ml of ethanol (6.11) while cooling the tubes. Mix the solution carefully. Allow the mixture to react for 10 min (ethanol + acetic acid anhydride ⇒ ethyl acetate).

Add 5 ml of water (6.1), mix and place the tube in a cold-water bath. The level of the water in the water bath shall exceed the level of the mixture in the tube during the following step.

Add 5 ml of 7,5 M KOH-solution (6.6.1), tighten the cap and mix. After a few minutes, add another 5-ml portion of 7,5 M KOH. Tighten the cap carefully and shake the tube vigorously. Allow the tubes to stand for at least 10 min until two clear phases have separated in the tube.

WARNING — It is important that the tube be cooled when the KOH-solution is being added, otherwise the ethyl acetate formed will evaporate.

Transfer the upper phase (ethyl acetate) into a test tube containing a small amount of dry sodium sulfate (6.12). Do this carefully so that no water phase is transferred.

Shake the tube and allow it to stand for 5 - 10 minutes. Transfer the clear solution to a septum vial and seal the vial.

9.4.2.4 Calibration

Calibrate the device using the monosaccharide standard solutions (6.2) containing the internal standard deoxy-galactose (6.13.1) Use the conditions recommended by the manufacturer or determine the optimum conditions empirically. The optimum conditions depend on the apparatus and the column.

9.4.2.5 Determination

Inject an aliquot of the test specimen prepared according to 9.4.2.3 into the instrument. Run the determination according to the manufacturer's instructions.

Check the integration of the chromatogram and the retention times for the different monosaccharides to be sure that they are adequate. If necessary, dilute the sample further until the concentration is within the calibration range. Run a new determination.

10 Calculation

Calculate the anhydrous content of each monosaccharide from using Formula 1:

$$X_i = \frac{A_i \cdot M_s \cdot k_i \cdot C_i}{A_s \cdot M} \quad (1)$$

where

X_i is the content of anhydrous monosaccharide *i* in the oven-dry sample, in mg per g;

A_i is the chromatographic area of monosaccharide *i*, in area units (i.e. signal·time);

M_s is the mass of the internal standard, in the undiluted sample, in mg;

C_i is the anhydrous factor for monosaccharide *i* (0,88 for xylose and arabinose, 0,90 for glucose, mannose and galactose);

A_s is the chromatographic area of the internal standard;

M is the oven-dry mass of the sample, in grams;

k_i is the calibration factor for monosaccharide *i*, (dimensionless), calculated as given in Formula 2

$$k_i = \frac{M_{i,c} \cdot A_{s,c}}{M_{s,c} \cdot A_{i,c}} \quad (2)$$

where

$M_{i,c}$ is the mass of monosaccharide *i* in calibration mixture, in mg;

$M_{s,c}$ is the mass of internal standard in calibration mixture, in mg;

$A_{i,c}$ is the chromatographic area of monosaccharide *i* in the calibration mixture, in area units (i.e., signal·time);

$A_{s,c}$ is the chromatographic area of the internal standard in the calibration mixture.

Report the results to the nearest whole number.

Calculate the relative content of each monosaccharide from Formula 3:

$$Y_i = \frac{100 \cdot X_i}{X_{tot}} \quad (3)$$

where

Y_i is the relative content of anhydrous monosaccharide *i*, in percent;

X_i is the content of anhydrous monosaccharide *i* in the oven-dry sample, in mg per g;

X_{tot} is the total content of anhydrous monosaccharides in the oven-dry sample, as the sum of the five monosaccharides, in mg per g.

Report the results to the first decimal place.

NOTE No correction factors were used to take into account the possible degradation of monosaccharides in standard solutions during acid hydrolysis. There is no definitive evidence to support the use of such factors. In fact, it is believed that monosaccharides in standard solutions can undergo more extensive degradation during hydrolysis than the polysaccharides in the original pulp. As such, the use of correction factors in the calculation of monosaccharide concentration could overestimate the carbohydrate composition and is not recommended.

11 Precision

The repeatability and reproducibility of each of the five monosaccharide tests were determined by conducting a round robin study with several types of pulp samples. A description of the samples used in this study and the round robin results are presented in Annex A.

Based on the results of the round robin study, a comparison was made between the IC/PAD and GC/FID procedures. On average, 4 laboratories used the IC/PAD procedure, and 3 laboratories used the GC/FID procedure. There were no statistical differences between the two procedures.

12 Test Report

The test report shall include the following information:

a) reference to this document i.e. ISO 21437:2020;

b) the date and place of testing;

c) all the information for complete identification of the sample;

d) the results expressed as the contents of the individual anhydrous monosaccharides in the oven-dry sample (in milligrams per gram);

e) the relative contents of the anhydrous monosaccharides (in percent);

f) the reference monosaccharides used for calibration;

g) any departure from the procedure described in this document, or any other circumstances which may have affected the result.

Annex A
(informative)

Precision

A.1 General

In June 2019, an international round robin study was performed in which seven laboratories from four different countries: Canada (2 laboratories); France; Japan (2 laboratories); and Sweden (2 laboratories) participated.

A total of five samples representing common types of mechanical and chemical pulps were included in the study. The samples were submitted to the participating laboratories for duplicate testing according to this document using either the IC/PAD or GC/FID procedure. The participants were also requested to extract one of the mechanical pulp samples (unbleached softwood TMP) with acetone and to perform the carbohydrate analysis on the extracted sample.

NOTE The average percent acetone extract of the unbleached softwood TMP sample was 3,9.

Repeatability and reproducibility data for arabinan, galactan, glucan, xylan, and mannan, for each type of sample, are shown in Tables A.1 to A.10. The calculations were made in accordance with ISO/TR 24498[5].

NOTE In a few cases, the results were considered as outliers and were not included in the precision statement.

NOTE The poor reproducibility of the arabinan, galactan, and mannan results for the bleached hardwood kraft sample is due to the very low levels of these monosaccharides in this sample.

The repeatability and reproducibility limits reported are estimates of the maximum difference which would be expected in 19 of 20 instances, when comparing two test results for material similar to those described under similar test conditions. These estimates may not be valid for different materials or different test conditions.

NOTE Repeatability and reproducibility limits are calculated by multiplying the repeatability and reproducibility standard deviations by 2,77, where $2,77 = 1,96 \sqrt{2}$.

A.2 Repeatability

Table A.1 — Estimation of the repeatability of the arabinan test

Type of pulp	Number of laboratories	Arabinan, mg/g Mean	Standard deviation S_r, mg/g	Coefficient of variation $C_{V,r}$, %	Repeatability limit r, mg/g
Unbleached softwood TMP (before acetone-extraction)	7	14,4	0,5	3,5	1,3
Unbleached softwood TMP (after acetone-extraction)	7	14,1	0,4	2,8	1,1
Unbleached hardwood RMP	7	6,5	0,3	4,6	0,7
Softwood BCTMP	7	12,0	0,5	4,2	1,3
Unbleached softwood kraft	7	6,4	0,2	3,1	0,6
Bleached hardwood kraft	7	1,5	0,2	13,3	0,6

Table A.2 — Estimation of the repeatability of the galactan test

Type of pulp	Number of laboratories	Galactan, mg/g Mean	Standard deviation S_r, mg/g	Coefficient of variation $C_{V,r}$, %	Repeatability limit r, mg/g
Unbleached softwood TMP (before acetone-extraction)	7	21,6	0,5	2,3	1,3
Unbleached softwood TMP (after acetone-extraction)	7	22,6	0,4	1,8	1,2
Unbleached hardwood RMP	7	6,4	0,6	9,4	1,7
Softwood BCTMP	7	21,9	0,3	1,4	0,9
Unbleached softwood kraft	7	4,4	0,2	4,5	0,5
Bleached hardwood kraft	7	0,8	0,1	12,5	0,2

Table A.3 — Estimation of the repeatability of the glucan test

Type of pulp	Number of laboratories	Glucan, mg/g Mean	Standard deviation S_r, mg/g	Coefficient of variation $C_{V,r}$, %	Repeatability limit r, mg/g
Unbleached softwood TMP (before acetone-extraction)	7	399,1	3,4	0,85	9,4
Unbleached softwood TMP (after acetone-extraction)	7	419,2	6,7	1,6	18,6
Unbleached hardwood RMP	6	414,8	3,0	0,72	8,4
Softwood BCTMP	7	457,4	3,5	0,77	9,8
Unbleached softwood kraft	7	743,5	9,3	1,3	25,7
Bleached hardwood kraft	6	756,0	13,2	1,7	36,6

Table A.4 — Estimation of the repeatability of the xylan test

Type of pulp	Number of laboratories	Xylan, mg/g Mean	Standard deviation S_r, mg/g	Coefficient of variation $C_{V,r}$, %	Repeatability limit r, mg/g
Unbleached softwood TMP (before acetone-extraction)	7	70,7	1,2	1,7	3,4
Unbleached softwood TMP (after acetone-extraction)	6	68,1	0,9	1,3	2,6
Unbleached hardwood RMP	6	151,7	1,5	0,99	4,2
Softwood BCTMP	7	55,1	1,3	2,4	3,5
Unbleached softwood kraft	6	81,4	0,6	0,74	1,7
Bleached hardwood kraft	7	168,3	5,4	3,2	14,9