
**Pulps — Determination of
carbohydrate composition**

Pâtes — Détermination de la composition des hydrates de carbone

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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.

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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://standards.iteh.ai/catalog/standards/sist/a7b237da-4253-45d9-8456-4a3d88900000/iso-21437-2020>

— 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 $40 \pm 0,5$ °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, NH₃ 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, KBH₄

6.7.1 Potassium borohydride solution

Dissolve 150 mg KBH₄ in 250 µl 13 M NH₃ 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, CH₃COOH, conc.

6.9 Acetic acid anhydride, conc.

6.10 1-methylimidazole

6.11 Ethanol, 95 - 99 %

6.12 Sodium sulfate, Na₂SO₄, 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 $105 \text{ °C} \pm 2 \text{ °C}$ 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 $300 \pm 10 \text{ mg}$ 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 $30 \pm 1 \text{ °C}$ 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 $120 \pm 3 \text{ °C}$ 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.