



BSI Standards Publication

**Pulps — Determination of lignin
content — Acid hydrolysis method**

National foreword

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**Pulps — Determination of lignin
content — Acid hydrolysis method**

*Pâtes — Détermination de la teneur en lignine — Méthode
d'hydrolyse acide*



Reference number
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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).

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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 main objective of measuring lignin in pulp is to assess the effect of a particular pulping or bleaching process on the degree of delignification. In chemical pulping, the goal is to remove lignin from wood with minimum degradation of the carbohydrates. The higher the level of residual lignin in any type of unbleached pulp, the greater the amount of bleaching chemicals that is applied in order to achieve a target brightness.

Comprehensive textbooks and reviews have been written on methods of lignin determination^{[1]-[3]}. This document specifies one such method, commonly used for the determination of the total lignin content of pulp. In this method, a pulp sample is treated with sulfuric acid, in a two-step (primary and secondary) hydrolysis process, to solubilize the carbohydrates. Most of the lignin remains insoluble at the end of the treatment and is filtered off, dried and weighed. This acid-insoluble lignin is also referred to as “Klason lignin”.

A small portion of lignin is dissolved during acid hydrolysis of the pulp. This so-called acid-soluble lignin is determined spectrophotometrically, from the UV absorbance at 205 nm of the filtrate from the acid-insoluble lignin determination^{[4]-[6]}. The total lignin content is determined as the sum of the acid-insoluble and acid-soluble lignin.

Two hydrolysis procedures are described in this document. In procedure A^{[7]-[9]}, the primary hydrolysis is performed with 72 % sulfuric acid at 30 °C for one hour, followed by dilution with water to 4 % sulfuric acid, and secondary hydrolysis in an autoclave at 120 °C for one hour. In procedure B^{[10][11]}, the primary hydrolysis is done at 15-20 °C for two hours, followed by secondary hydrolysis at 3 % sulfuric acid in a water bath at 100 °C for four hours. In procedure A, the use of 4 % sulfuric acid, instead of 3 %, for secondary hydrolysis has no impact on the lignin analysis and is accepted when both lignin and carbohydrates need to be analysed in the same sample.

Both procedures have been shown to give the same results; thus, either one can be used for determining acid-insoluble lignin. However, procedure A is considerably more rapid, and the use of an autoclave allows multiple samples to be hydrolysed simultaneously with minimum supervision. As such, it is now more commonly used in laboratories equipped with an autoclave. It is therefore the preferred method and should be used when analysis of carbohydrates is required in addition to the determination of lignin.

Pulps — Determination of lignin content — Acid hydrolysis method

WARNING — This method involves the use of hazardous chemicals. Care should be taken to ensure that the relevant precautions are taken.

1 Scope

The method is applicable to unbleached, bleached and semi-bleached wood pulp with a lignin content above 1 %. It is not generally intended for fully bleached chemical pulp, because the lignin content in these pulps is too low to be determined accurately.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 1762](#), *Paper, board, pulps and cellulose nanomaterials — Determination of residue (ash content) on ignition at 525 °C*

[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

lignins

class of complex, organic macromolecules, containing aromatic sub-units, that play a key role in the formation of cell walls in wood and bark, conferring mechanical strength and rigidity to cell walls and to plants as a whole

Note 1 to entry: Lignin is the main non-carbohydrate constituent of wood.

3.2

acid-insoluble (Klason) lignin

residue after treating wood or pulp with sulfuric acid in a two-step hydrolysis procedure to solubilize the carbohydrates into monosaccharides

3.3

acid-soluble lignin

portion of *lignin* (3.1) which is soluble during the acid-insoluble lignin determination

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.

Obtain a representative sample of pulp equivalent to about 10 g moisture-free pulp. Air dry the pulp and disintegrate in a household blender, or grind in a Wiley mill to pass a No. 20 (0,85 mm) screen. Groundwood and high yield pulps containing a significant amount of resins shall be extracted with acetone ([6.3](#)) according to [ISO 14453](#) before testing.

NOTE Resins, if not extracted from the pulp prior to analysis, would remain insoluble in acid and be weighed as lignin.

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.

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

8 Test Specimens

Weigh ([5.6](#)) a test specimen, equivalent to 300 mg to 1,0 g of oven-dried mass of pulp to the nearest 0,1 mg (see NOTE) and transfer to a 200 ml beaker. Make sure that the test specimens taken are representative of the sample received.

The mass of the test specimen is such as to provide a minimum of 20 mg of lignin in the residue remaining after acid hydrolysis, for accurate weighing. The following can be used as a guide to the amount of pulp that should be selected for analysis, based on the lignin content:

- >10 % lignin: 300 mg pulp
- 5-10 % lignin: 500 mg pulp
- <5 % lignin: 1,0 g pulp

NOTE The amount of pulp selected for analysis also depends on whether hydrolysis procedure A or hydrolysis procedure B is used.

9 Procedure

Run the entire procedure in duplicate.

9.1 Hydrolysis

9.1.1 General

Two hydrolysis procedures are described in this Standard. In procedure A[[7](#)]-[[9](#)], the primary hydrolysis is performed at 30 °C for 1 h, followed by secondary hydrolysis in an autoclave at 120 °C for 1 h. In procedure B[[10](#)][[11](#)], the primary hydrolysis is performed at 15 - 20 °C for 2 h, followed by secondary hydrolysis in a water bath at 100 °C for 4 h .

NOTE Boiling under reflux is not allowed if acid-soluble lignin is to be determined in the solution (TAPPI T222, Section 9.4, NOTE 6). This is due to the possible contribution of furfural compounds to the acid-soluble lignin, if not removed in this step (Ref.2, p. 192).

Both procedures have been shown to give the same results[[7](#)]; thus either one can be used for determining acid-insoluble lignin. However, procedure A is considerably more rapid, and the use of

an autoclave allows multiple samples to be hydrolysed simultaneously with minimum supervision. As such, it is now more commonly used in laboratories equipped with an autoclave.

NOTE Procedure A is therefore the preferred method and is used when analysis of carbohydrates is required, in addition to the determination of lignin.

9.1.2 Hydrolysis procedure A

Hydrolysis procedure A is based on the use of 300 mg of pulp. This procedure is more commonly used in laboratories equipped with an autoclave, and is similar to that described in other methods[7]-[9] in situations in which analysis of carbohydrates is required, in addition to analysis of lignin.

For larger pulp specimens, the acid concentrations for both primary and secondary hydrolysis must remain the same as those used for 300 mg of pulp; thus the volume of solutions must be adjusted accordingly.

Add 3,0 ml of 72 % sulfuric acid (6.2) to the test specimen in the beaker. Add the acid gradually in small increments while stirring and macerating the material with a glass rod. To avoid losses, ensure that no material is sticking to the glass rod when it is removed.

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 minutes to facilitate wetting and absorption.

Place the beaker in a water bath adjusted to $30 \pm 1,0$ °C for 1 h. Stir occasionally.

Add 84,0 ml of water. Mix, cover the beaker with aluminium foil and place it in an autoclave at (120 ± 3) °C for 1 h. Allow the insoluble lignin to settle and for the beaker and contents to cool to approximately 80 °C.

NOTE In some cases, the lignin requires an overnight or a longer period to settle, especially if it is finely dispersed.

9.1.3 Hydrolysis procedure B

Hydrolysis procedure B is based on the use of 1,0 g of pulp, and is similar to that described in other methods[10][11]. This procedure is also used in some laboratories.

NOTE Hydrolysis procedure B is not used when carbohydrate analysis is also required.

For smaller pulp specimens, the acid concentrations for both primary and secondary hydrolysis must remain the same as those used for 1,0 g of pulp; thus the solution volumes shall be adjusted accordingly.

Place the beaker with the test specimen in a water bath (5.2) at 20 ± 1 °C and add gradually 20,0 ml of 72 % sulfuric acid (6.2), maintaining a reaction temperature of 20 ± 1 °C. Stir thoroughly with a glass rod for about 1 min. To avoid losses, ensure that no material is sticking to the glass rod when it is removed.

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.

Keep the beaker in the bath for 120 ± 10 min with occasional stirring.

Transfer the material quantitatively from the beaker into a 1 000 ml Erlenmeyer flask and dilute with water to 750 ml.

Boil the solution for 4 h, maintaining a constant volume of solution by frequent addition of hot (80-90 °C) water. Allow the insoluble lignin to settle and for the flask and contents to cool to approximately 80 °C.

NOTE In some cases, the lignin requires an overnight or a longer period to settle, especially if it is finely dispersed.

9.2 Filtration

Without stirring the insoluble lignin residue, decant or siphon off the supernatant solution through a pre-weighed filtering crucible (5.1) placed on a 250 ml filtering flask (5.1) (hydrolysis procedure A) or a 1 000 ml filtering flask (5.1) (hydrolysis procedure B). Proceed to filtration procedure A if hydrolysis procedure A is used, or to filtration procedure B if hydrolysis procedure B is used.

Filtration procedure A: 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. Allow to cool to room temperature and fill up to the mark with water (6.1). This filtrate will be used for the acid-soluble lignin determination.

NOTE Dilution to precisely 250 ml also allows accurate quantification of carbohydrates in the filtrate, if required.

Filtration procedure B: Proceed as in filtration procedure A, but transfer the filtrate to a 1 l volumetric flask, wash the precipitate with 2 × 100 ml warm water (6.1), and add the washings to the volumetric flask. Allow to cool to room temperature and fill up to the mark with water. This filtrate will be used for the acid-soluble lignin determination.

9.3 Acid-insoluble lignin determination

Quantitatively transfer the insoluble lignin, from 9.1.2 or 9.1.3, to the **pre-weighed** filtering crucible (5.1) using hot water (6.1) and a rod with rubber policeman. Wash the lignin with hot water (6.1) until the pH of the filtrate is in the range of 6-7, as confirmed with the use of a pH indicator paper or pH meter. Discard this filtrate.

Dry the crucible with lignin in an oven (5.5) at 105 ± 2 °C to constant weight. Cool in a desiccator and weigh with an analytical balance(5.6).

If a correction for ash is required, transfer the lignin from the previous step to a small pre-weighed platinum or porcelain crucible and determine the ash content according to [ISO 1762](#).

9.4 Acid-soluble lignin determination

Use the filtrate from the acid-insoluble lignin determination (9.2) as a test specimen.

Measure the absorbance of the filtrate at 205 nm in the 1,0 cm pathlength cuvette of the spectrophotometer (5.7). Use 4 % sulfuric acid (hydrolysis procedure A) or 3 % sulfuric acid (hydrolysis procedure B), as a blank. If necessary, dilute the filtrate with 4 % sulfuric acid (hydrolysis procedure A) or 3 % sulfuric acid (hydrolysis procedure B), such that its absorbance is in the range of 0,2-0,7 AU.

NOTE Degradation products from carbohydrates can contribute to the measured acid-soluble lignin[6]. However, this contribution is generally small and can be neglected.

NOTE In the round robin study ([Annex A](#)), labs using the autoclave (hydrolysis procedure A) reported levels of acid-soluble lignin that were on average 0,2 % higher than those obtained when using the water bath (hydrolysis procedure B). This could indicate that furfural compounds are not completely removed when using the autoclave, due to the fact that the beaker is covered with aluminium foil during the secondary hydrolysis. However, since these differences are relatively small, they are unlikely to have a significant impact on the total lignin results.

10 Calculation

Calculate the mean of duplicate determinations

$$\text{Acid-insoluble lignin, \%} = \frac{m \cdot 100}{M}$$

where

m is the mass of insoluble lignin from 9.3, g;

M is the mass of oven-dried pulp test specimen, g.

$$\text{Acid-soluble lignin in filtrate from 9.2, g/l} = \frac{A \cdot D}{110}$$

where

A is the absorbance at 205 nm with a 1 cm pathlength cuvette;

D is the dilution factor required to bring the absorbance in the range of 0,2-0,7 AU.

The factor of 110 represents the absorptivity of lignin (l/g.cm) at 205 nm[4]-[6].

NOTE 1 The absorption coefficient for determining the acid-soluble lignin in many wood species at 205 nm was found, on average, to be 110 l/g.cm (5,6). In spite of the fact that no similar studies have been conducted on various types of pulp, an absorption coefficient of 110 l/g.cm is also applied for the determination of acid-soluble lignin in pulp⁽⁴⁾.

NOTE 2 The extinction coefficient of lignin depends not only on the type of wood species, but also on the pulping process and yield[12].

The acid-soluble lignin is usually expressed as a percentage of pulp, as follows:

$$\text{Acid-soluble lignin, \%} = \frac{ASL \cdot V \cdot 100}{1\,000 \cdot M}$$

where

ASL is the acid-soluble lignin in filtrate, g/l;

V is the total volume of filtrate, i.e., 250 ml (hydrolysis procedure A) or 1 000 ml (hydrolysis procedure B).

$$\text{Total lignin, \%} = \text{Acid-insoluble lignin} + \text{acid-soluble lignin}$$

11 Precision

The repeatability and reproducibility of the acid-insoluble, acid-soluble and total lignin 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 hydrolysis procedures A and B for the acid-insoluble lignin, acid-soluble lignin, and total lignin. On average, 6 laboratories used hydrolysis procedure A, and 3 laboratories used hydrolysis procedure B. There were no statistical differences between the two procedures.

12 Test Report

The test report shall include the following:

- reference to this document i.e. [ISO 21436:2020](#);
- the date and place of testing;
- all the information for complete identification of the sample;
- the percent of acid-insoluble, acid-soluble, and total lignin, expressed as indicated in [Clause 10](#);
- any unusual features observed in the course of the test;

- f) any departure from the procedures 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 nine laboratories from six different countries: Brazil; Canada (2 laboratories); China; France; Japan (2 laboratories); and Sweden (2 laboratories) participated. In the case of one laboratory, the analyses were performed on some of the samples using both hydrolysis methods A and B; these results were included as representing two laboratories.

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 testing according to this document using either hydrolysis method A or B. The participants were also requested to extract one of the mechanical pulp samples (unbleached softwood TMP) with acetone and to perform the lignin analysis on the extracted sample.

NOTE The percent acetone extract of the unbleached softwood TMP sample was 3,9, based on the average values reported by six laboratories.

NOTE In spite of the fact that this method is not generally applicable to fully bleached kraft pulp, it was decided to include one such pulp in the round robin study. The intent was to assess whether degraded acid-soluble lignin and carbohydrate degradation products that may condense under acidic conditions, forming insoluble polymeric material, could potentially contribute to the lignin measurement. As indicated by the data in [Tables A.1 to A.6](#), any such contribution, if at all present, could be ignored.

Repeatability and reproducibility data for acid-insoluble lignin; acid-soluble lignin; and total lignin for each type of sample are shown in [Tables A.1 to A.6](#). The calculations were made in accordance with [ISO/TR 24498 \[13\]](#).

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

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 acid-insoluble lignin test

Type of pulp	Number of laboratories	Mean Acid-insoluble lignin %	Standard deviation S_r , %	Coefficient of variation $C_{V,r}$, %	Repeatability limit r , %
Unbleached softwood TMP (before acetone-extraction)	10	30,9	0,4	1,3	1,0
Unbleached softwood TMP (after acetone-extraction)	10	28,1	0,4	1,4	1,0
Unbleached hardwood RMP	8	23,2	0,3	1,3	0,7
Softwood BCTMP	10	25,7	0,3	1,1	0,9
Unbleached softwood kraft	9	3,4	0,3	8,8	0,7
Bleached hardwood kraft	9	0,6	0,1	16,7	0,3

Table A.2 — Estimation of the repeatability of the acid-soluble lignin test

Type of pulp	Number of laboratories	Mean Acid-soluble lignin %	Standard deviation S_r , %	Coefficient of variation $C_{V,r}$, %	Repeatability limit r , %
Unbleached softwood TMP (before acetone-extraction)	10	0,5	0,1	20,0	0,2
Unbleached softwood TMP (after acetone-extraction)	10	0,4	0,0	0,0	0,1
Unbleached hardwood RMP	9	3,1	0,2	6,4	0,4
Softwood BCTMP	10	0,9	0,0	0,0	0,1
Unbleached softwood kraft	9	0,5	0,0	0,0	0,1
Bleached hardwood kraft	9	0,4	0,0	0,0	0,0

Table A.3 — Estimation of the repeatability of the total lignin test

Type of pulp	Number of laboratories	Mean total lignin %	Standard deviation $S_r, \%$	Coefficient of variation $C_{V,r}, \%$	Repeatability limit $r, \%$
Unbleached softwood TMP (before acetone-extraction)	10	31,4	0,4	1,3	1,1
Unbleached softwood TMP (after acetone-extraction)	10	28,5	0,4	1,4	1,0
Unbleached hardwood RMP	8	26,2	0,3	1,1	0,9
Softwood BCTMP	10	26,6	0,3	1,1	0,9
Unbleached softwood kraft	9	3,8	0,2	5,3	0,6
Bleached hardwood kraft	9	1,1	0,1	9,1	0,3

Table A.4 — Estimation of the reproducibility of the acid-insoluble lignin test

Type of pulp	Number of laboratories	Mean Acid-insoluble lignin %	Standard deviation $S_R, \%$	Coefficient of variation $C_{V,R}, \%$	Reproducibility limit $R, \%$
Unbleached softwood TMP (before acetone-extraction)	9	30,8	0,4	1,3	1,2
Unbleached softwood TMP (after acetone-extraction)	10	28,1	0,8	2,8	2,1
Unbleached hardwood RMP	10	23,0	0,9	3,9	2,5
Softwood BCTMP	10	25,7	0,4	1,6	1,1
Unbleached softwood kraft	9	3,4	0,5	14,7	1,4
Bleached hardwood kraft	9	0,6	0,4	66,7	1,0

Table A.5 — Estimation of the reproducibility of the acid-soluble lignin test

Type of pulp	Number of laboratories	Mean Acid-soluble lignin %	Standard deviation $S_R, \%$	Coefficient of variation $C_{V,R}, \%$	Reproducibility limit $R, \%$
Unbleached softwood TMP (before acetone-extraction)	9	0,5	0,1	20,0	0,4
Unbleached softwood TMP (after acetone-extraction)	10	0,4	0,2	50,0	0,5
Unbleached hardwood RMP	9	3,1	0,4	12,9	1,2
Softwood BCTMP	9	0,7	0,2	28,6	0,5
Unbleached softwood kraft	9	0,5	0,1	20,0	0,4
Bleached hardwood kraft	9	0,4	0,2	50,0	0,5

Table A.6 — Estimation of the reproducibility of the total lignin test

Type of pulp	Number of laboratories	Mean total lignin %	Standard deviation $S_R, \%$	Coefficient of variation $C_{V,R}, \%$	Reproducibility limit $R, \%$
Unbleached softwood TMP (before acetone-extraction)	9	31,2	0,5	1,6	1,4
Unbleached softwood TMP (after acetone-extraction)	10	28,5	0,9	3,2	2,4
Unbleached hardwood RMP	9	26,2	0,9	3,4	2,4
Softwood BCTMP	9	26,3	0,4	1,5	1,0
Unbleached softwood kraft	9	3,8	0,5	13,2	1,5
Bleached hardwood kraft	9	1,1	0,5	45,5	1,4

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