INTERNATIONAL STANDARD

ISO 24196

First edition
2022-06

Lignins — Determination of lignin content in kraft lignin, soda lignin and hydrolysis lignin

STANDARDSISO.COM : Click to view the full PDF of ISO 24196:2022

Reference number
ISO 24196:2022(E)

© ISO 2022

STANDARDSISO.COM : Click to view the full PDF of ISO 24196:2022

COPYRIGHT PROTECTED DOCUMENT

© ISO 2022

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

STANDARDSISO.COM : Click to view the full PDF of ISO 24196:2022

© ISO 2022 – All rights reserved

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.

STANDARDSISO.COM : Click to view the full PDF of ISO 24196:2022

© ISO 2022 – All rights reserved

Introduction

There is a rapidly-growing worldwide interest in developing novel applications for lignin as a replacement for fossil-based raw materials in products including carbon fibre, adhesives, thermoplastics, resins, composites, and various chemicals. In addition, the use of lignin in these and other applications will offload recovery boilers in pulp mills, allowing more efficient recovery of pulping chemicals and increased pulp production. These benefits translate into reduced environmental impact and improved sustainability owing to the use of renewable materials.

In order to ensure harmonization of testing practices among lignin producers and to facilitate trade, the use of international standard methods is needed to characterize the lignin raw material for a wide range of properties such as general composition, functional groups, molecular weight distribution, particle size, structural features, and thermal behaviour and stability.

The total lignin content provides an indication of the purity of the lignin isolated from the kraft pulping process (kraft lignin) or the soda pulping process (soda lignin), or that obtained by hydrolysis of biomass (hydrolysis lignin).

The method described in this document is based on that described in other publications[1][2][3]. Although the principle is similar to that described in ISO 21436[4] and other related methods[5][6][7] for the determination of lignin in pulp, the properties and end-use applications of lignin, as well as several steps in the testing procedure, including sampling, sample preparation, and others, are different from those of pulp.

STANDARDSISO.COM : Click to view the full PDF of ISO 24196:2022

© ISO 2022 – All rights reserved

STANDARDSISO.COM : Click to view the full PDF of ISO 24196:2022

Lignins — Determination of lignin content in kraft lignin, soda lignin and hydrolysis lignin

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

1 Scope

This document describes a method for the determination of lignin content in kraft, soda, and hydrolysis lignin.

The method is applicable to lignin isolated from a kraft pulping process, a soda pulping process, or lignin obtained by hydrolysis of biomass.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

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

- ISO Online browsing platform: available at https://www.iso.org/obp
- IEC Electropedia: available at https://www.electropedia.org/

3.1
lignins
class of complex organic macromolecules, containing aromatic sub-units, that plays a key role in the formation of cell walls in wood and bark, conferring mechanical strength and rigidity to the cell walls and to plants as a whole

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

3.2
kraft lignin
depolymerized and chemically modified lignin isolated from a kraft pulping process, such as that originating from kraft black liquor

3.3
soda lignin
depolymerized and chemically modified lignin isolated from a soda pulping process, such as that originating from soda liquor

3.4
hydrolysis lignin
lignin produced for commercial applications by conversion of biomass, through enzymatic or acid hydrolysis, into sugars and lignin streams, followed by separation of the lignin fraction

3.5
biomass
biological material derived from living, or previously living organisms, such as wood, agricultural crops and other plant-based biodegradable material

STANDARDSISO.COM : Click to view the full PDF of ISO 24196:2022

© ISO 2022 – All rights reserved

3.6
acid-insoluble lignin
klason lignin
residue after treating wood or pulp with sulfuric acid in a two-step hydrolysis procedure to solubilize the carbohydrates into monosaccharides

3.7
acid-soluble lignin
portion of lignin that is soluble during the acid-insoluble lignin determination

4 Principle

A lignin sample is treated with sulfuric acid in a two-step (primary and secondary) hydrolysis process to dissolve the carbohydrates. The residue after hydrolysis is filtered off, dried, and weighed, and referred to as acid-insoluble lignin (or Klason lignin). A small amount of lignin is dissolved during acid hydrolysis. This so-called acid-soluble lignin is determined by measuring the absorbance at 205 nm of the filtrate from the acid-insoluble lignin determination. The total lignin content is determined as the sum of the acid-insoluble lignin and acid-soluble lignin.

According to Reference [8], proteins present in plant tissue condense extensively with lignin during the sulfuric acid treatment, imparting extra mass to the measured Klason lignin. Since the enzymes used to produce hydrolysis lignin consist largely of proteins, a correction to the measured acid-insoluble lignin in hydrolysis lignin shall be made to account for this additional material. The amount of co-condensed protein in acid-insoluble lignin can be estimated by multiplying its nitrogen content by a factor of 6,25[8]. Nitrogen analysis can be performed using the traditional Kjeldahl method or with an automated nitrogen analyser, provided that the results have been properly validated.

NOTE The secondary hydrolysis is performed with 4 % sulfuric acid instead of 3 % as described in other methods for the determination of lignin in wood and pulp[5][6]. The use of 4 % sulfuric acid for secondary hydrolysis instead of 3 % has no impact on the lignin analysis and is accepted when both lignin and carbohydrates need to be analysed in the same sample.

5 Apparatus

5.1 Filtration equipment

5.1.1 Filtering flask, 250 ml.

5.1.2 Gooch filtering crucible, fritted glass, medium or fine porosity, 30 ml; adapter for the filtering crucible, siphon tube (optional).

NOTE 1 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. 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.

NOTE 2 Other types of filtering crucibles such as alundum or porous porcelain crucibles lined with a mat of fine fibres can also be used.

5.2 Constant temperature water bath, capable of maintaining a temperature of (30 ± 1) °C.

5.3 Autoclave, capable of maintaining a temperature of (120 ± 3) °C.

5.4 Drying oven, conduction type, maintained at (105 ± 2) °C.

A convection oven shall not be used, as this could lead to increased flare-ups and fire hazard, as well as loss of sample due to material being ejected from the crucible.

STANDARDSISO.COM : Click to view the full PDF of ISO 24196:2022

© ISO 2022 – All rights reserved

5.5 **Analytical balance,** accurate to 0,1 mg.

5.6 Spectrophotometer, UV-visible, diode array or simple wavelength, with high purity quartz cuvettes of pathlength 1 cm.

6 Reagents

6.1 Water, of high purity, distilled or deionized.

6.2 Sulfuric acid, 72 % (720 g/kg, specific gravity 1,633 8 at 20 °C). 72 % sulfuric acid is available commercially. It can also 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 sulfuric acid solution to 1,633 8 with the use of a hydrometer by careful addition of concentrated sulfuric acid or water.

6.3 Petroleum ether, ACS or reagent grade.

7 Sampling

Obtain a representative sample of lignin equivalent to about 2 g to 3 g on an air-dry basis. Report the origin of the sample and the sampling procedure. In particular, if the sample is first ground to ensure its homogeneity, and/or a sieving step is required in order to obtain samples with a uniform particle size or narrow particle size distribution, this shall be reported.

Lignin samples can contain a significant amount of resins and shall be extracted with petroleum ether (6.3) before testing. The percentage resin in the sample and the extraction method shall be included in the test report.

Extraction with petroleum ether should be carried out by a method similar to that described in ISO 14453[9], using petroleum ether instead of acetone.

Although acetone is an effective solvent for extracting resin, it cannot be used here since it will also dissolve part of the lignin.

NOTE Resins, if not extracted from lignin samples prior to analysis, will remain insoluble in acid and be weighed as acid-insoluble lignin.

STANDARDSISO.COM : Click to view the full PDF of ISO 24196:2022

8 Drying

Prior to drying to complete dryness, lignin samples shall be air-dried to over 75 % (g/100 g) solids. This step is necessary in order to minimize the extent of lignin degradation reactions and the drying period.

Determine the dry matter content of the lignin by drying a 2 g to 3 g specimen in an oven (5.4) at (105 ± 2) °C initially for 2 h, and then for additional 1-h periods up to a maximum of 7 h, until the difference in mass between two successive dryings in a desiccator, does not exceed 0,5 % mass fraction (g/100 g) of the test piece before drying.

9 Test specimens

Weigh (5.5) a test specimen, equivalent to about 100 mg of oven-dried lignin, to the nearest 0,1 mg and transfer to a 50 ml to 100 ml beaker.

10 Procedure

10.1 General

Carry out the entire procedure in duplicate.

10.2 Hydrolysis

Add 1,0 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 lignin samples 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 water bath (5.2) adjusted to (30 ± 1) °C for 1 h. Stir occasionally.

Add 28,0 ml of water (6.1). Mix, cover the beaker with aluminium foil, and place it in an autoclave (5.3) at (120 ± 3) °C for 1 h. Allow the insoluble lignin to settle and the beaker and contents to cool to approximately 80 °C.

NOTE If the lignin is finely dispersed, it can require overnight or longer to settle.

10.3 Filtration

Without stirring the insoluble lignin residue, decant or siphon off the supernatant solution through a pre-weighed filtering crucible (5.1.2) placed on a 250 ml filtering flask (5.1.1).

Transfer the filtrate to a 250 ml volumetric flask. Wash the precipitate on the filtering crucible with 2 × 30 ml warm water (6.1) and add the washings to the volumetric flask. Rinse the filtering flask with a small amount of water and add the rinsings to the 250 ml 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.

NOTE Dilution to precisely 250 ml also allows accurate quantification of carbohydrates in the filtrate, if required, as described in ISO 24215[10].

10.4 Acid-insoluble lignin determination

Quantitatively transfer the insoluble lignin, from 10.2, to the pre-weighed filtering crucible (5.1.2) using hot water (6.1) and a rod with rubber policeman, combining with the precipitate from filtration of the supernatant in 10.3. Wash the precipitate on the filtering crucible from 10.3 with hot water (6.1) until the pH of the filtrate is in the range of 6 to 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 (5.4) at (105 ± 2) °C to constant weight. Cool in a desiccator and weigh (5.5).

10.5 Acid-soluble lignin determination

Use the filtrate from 10.3 as a test specimen.

Measure the absorbance of the filtrate at 205 nm in the 1,0 cm pathlength cuvette of the spectrophotometer (5.6). Use 4 % sulfuric acid as a blank. If necessary, dilute the filtrate with 4 % sulfuric acid such that its absorbance is in the range of 0,2 AU to 0,7 AU.

NOTE 1 Degradation products from carbohydrates can contribute to the measured acid-soluble lignin[11]. However, this contribution is generally small and can be neglected.

STANDARDSISO.COM : Click to view the full PDF of ISO 24196:2022

© ISO 2022 – All rights reserved

NOTE 2 In a round robin study for determination of lignin content in pulp (in ISO 21436:2020 Annex A), laboratories using the autoclave (Hydrolysis procedure A in ISO 21436:2020, 9.12) reported levels of acid-soluble lignin that were on average 0,2 % higher than those obtained when using the water bath (alternative to using an autoclave, Hydrolysis procedure B in ISO 21436:2020 9.13). This can 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 likely have no significant impact on the total lignin results.

11 Calculation

Calculate the mean of duplicate determinations.

Acid-insoluble lignin is calculated as follows:

$$L_{\mathrm{AI}} = \frac{m \times 100}{M}$$

where

L_{AI} is the acid-insoluble lignin, in % (g/100 g of lignin);

m is the mass of insoluble lignin, g; corrected for protein content, if necessary;

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

The acid-soluble lignin in the filtrate from 10.5 is calculated as follows:

$$L_{\mathrm{AS,F}} = \frac{A \times D}{110}$$

where

$L_{\mathrm{AS,F}}$ is the acid-soluble lignin in filtrate, in g/l;

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 AU to 0,7 AU. The factor of 110 represents the absorptivity of lignin (l/g·cm) at 205 nm[7][11][12].

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[11][12]. 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[7]. The same absorption coefficient of 110 l/g·cm is also used here for the determination of acid-soluble lignin in the various types of lignins within the scope of this document.

NOTE 2 The absorption coefficient of lignin depends not only on the type of wood species, but also on the pulping process and yield[13].

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

$$L_{\mathrm{AS}} = \frac{L_{\mathrm{AS,F}} \times V \times 100}{1\,000 \times M}$$

where

L_{AS} is the acid-soluble lignin, g/100 g;

$L_{\mathrm{AS,F}}$ is the acid-soluble lignin in filtrate, g/l;

V is the total volume of filtrate, ml, i.e. 250 ml.

STANDARDSISO.COM : Click to view the full PDF of ISO 24196:2022

Total lignin, % (g/100 g) = acid-insoluble lignin + acid-soluble lignin

12 Precision

The precision of the method was determined by conducting a round robin study with several types of lignin samples. A description of the samples used in this study, and the repeatability and reproducibility results are presented in Annex A.

13 Test report

The test report shall include the following:

a) a reference to this document, i.e. ISO 24196:2022;

b) the date and place of testing;

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

d) the percent of acid-insoluble, acid-soluble, and total lignin, expressed as indicated in Clause 11;

e) percentage resin in the sample and the extraction method used;

f) any unusual features observed in the course of the test;

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

STANDARDSISO.COM : Click to view the full PDF of ISO 24196:2022

© ISO 2022 – All rights reserved

Annex A
(informative)

Precision

A.1 General

In June 2020 an international round robin study was performed in which 10 laboratories from seven different countries: Brazil, Canada (two laboratories), China, France; Japan (two laboratories), Sweden (two laboratories) and USA participated.

A total of four samples representing different types of lignins were included in the study, including a softwood kraft lignin, acid form; a hardwood kraft lignin, acid form; a hydrolysis hardwood lignin, washed; and a soda lignin, acid form. The samples were submitted to the participating laboratories for testing according to this document.

The participants were also requested to extract the softwood kraft lignin sample with petroleum ether and to perform the lignin analysis on the extracted sample.

NOTE 1 The percent petroleum ether extract of the unbleached softwood lignin sample was 1,2, based on the average values reported by six laboratories.

Participants were also asked to determine the nitrogen content of the hydrolysis hardwood and soda lignin samples.

NOTE 2 The percent nitrogen content of the hydrolysis hardwood and soda lignin samples were 0,37 and 0,78, respectively, based on the average values reported by six laboratories.

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 acid-insoluble, acid-soluble, and total lignin, as well as the standard deviation and repeatability/reproducibility limits are all expressed in % (g/100 g) of lignin. The coefficient of variation is expressed as a percentage of the standard deviation. The calculations were made according to ISO/TS 24498[14].

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 might not be valid for different materials or different test conditions.

NOTE 3 Repeatability and reproducibility limits are calculated by multiplying the repeatability and reproducibility standard deviations by 2,77, where 2,77 = 1,96 √2.

STANDARDSISO.COM : Click to view the full PDF of ISO 24196:2022

A.2 Repeatability

Table A.1 — Estimation of the repeatability of the acid-insoluble lignin test

Type of lignin	Number of laboratories	Mean Acid-insoluble lignin %	Standard deviation S_r, %	Coefficient of variation $C_{V,r}$, %	Repeatability limit r, %
Softwood kraft	9	90,8	1,0	1,1	2,7
Softwood kraft, extracted	8	88,0	0,6	0,68	1,8
Hardwood kraft	10	70,3	1,2	1,7	3,5

Table A.1 *(continued)*

Type of lignin	Number of laboratories	Mean Acid-insoluble lignin %	Standard deviation S_r, %	Coefficient of variation $C_{V,r}$, %	Repeatability limit r, %
Hydrolysis hardwood	10	55,5	1,5	2,7	4,3
Soda	10	82,6	2,4	2,9	6,6

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

Type of lignin	Number of laboratories	Mean Acid-soluble lignin %	Standard deviation S_r, %	Coefficient of variation $C_{V,r}$, %	Repeatability limit r, %
Softwood kraft	10	2,8	0,2	7,1	0,4
Softwood kraft, extracted	8	3,3	0,1	3,0	0,4
Hardwood kraft	10	6,8	0,2	2,9	0,4
Hydrolysis hardwood	10	3,6	0,2	5,6	0,7
Soda	10	6,2	0,5	8,1	1,4

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

Type of lignin	Number of laboratories	Mean total lignin %	Standard deviation S_r, %	Coefficient of variation $C_{V,r}$, %	Repeatability limit r, %
Softwood kraft	10	92,8	0,9	0,97	2,5
Softwood kraft, extracted	8	91,4	0,6	0,66	1,7
Hardwood kraft	10	77,1	1,2	1,6	3,3
Hydrolysis hardwood	10	59,0	1,4	2,4	3,8
Soda	10	88,8	2,3	2,6	6,5

A.3 Reproducibility

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

Type of lignin	Number of laboratories	Mean Acid-insoluble lignin %	Standard deviation S_R,%	Coefficient of variation $C_{V,R}$, %	Reproducibility limit R, %
Softwood kraft	9	90,8	2,1	2,3	5,7
Softwood kraft, extracted	8	88,0	4,0	4,5	11,1
Hardwood kraft	10	70,3	3,0	4,3	8,2
Hydrolysis hardwood	10	55,5	4,2	7,6	11,8
Soda	10	82,6	4,9	5,9	13,6

STANDARDSISO.COM : Click to view the full PDF of ISO 24196:2022

© ISO 2022 – All rights reserved