
वस्त्रादि —  सन के रेशों में घटकों का
निर्धारण

(ISO 5773 : 2023,  सशाधित)

Textiles —  Determination of
Components in Flax Fibres

(ISO 5773 : 2023, MOD)

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NATIONAL FOREWORD

This Indian Standard which is modified adoption of ISO 5773 : 2023 'Textiles — Determination of components in flax fibres' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on the recommendation of the Chemical Methods of Test Sectional Committee and approval of the Textiles Division Council.

The text of ISO Standard has been approved as suitable for publication as an Indian Standard without deviations. Certain conventions are, however, not identical to those used in Indian Standards. Attention is particularly drawn to the following:

- a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'; and
- b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In this adopted standard reference appears to certain International Standards for which Indian Standards also exist. The corresponding Indian Standards which are to be substituted in their respective places are listed below along with their degree of equivalence for the editions indicated:

<i>International Standard</i>	<i>Corresponding Indian Standard</i>	<i>Degree of Equivalence</i>
ISO 3696 Water for analytical laboratory use — Specification and test methods	IS 1070 : 2023 Reagent grade water — Specification (<i>fourth revision</i>)	Technically Equivalent
ISO 4793 Laboratory sintered (fritted) filters — Porosity grading, classification and designation	IS 12305 : 2018/ISO 4793 : 1980 Laboratory sintered (fritted) filters — Porosity grading, classification and designation (<i>first revision</i>)	Identical

The committee has reviewed the provisions of the following International Standard referred in this adopted standard and has decided that it is acceptable for use in conjunction with this standard:

<i>International/Other Standard</i>	<i>Title</i>
ISO 1130 : 1975	Textile fibres — Some methods of sampling for testing

In reporting the result of a test or analysis made in accordance with this standard, if the final value; observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 2022 'Rules for rounding off numerical values (*second revision*)'.

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*Indian Standard***TEXTILES — DETERMINATION OF COMPONENTS IN FLAX FIBRES****(ISO 5773 : 2023, MOD)****1 Scope**

This document specifies the test methods for the quantitative analysis of cellulose, hemicellulose, lignin, pectin, fat and wax content in flax fibres.

This document is applicable to flax fibres and can be used as a reference for testing other bast fibres.

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 1130, *Textile fibres — Some methods of sampling for testing*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 4793, *Laboratory sintered (fritted) filters — Porosity grading, classification and designation*

3 Terms and definitions

No terms and definitions are listed in this document.

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 <https://www.electropedia.org/>

4 Principle

Flax fibres were treated physically and chemically to extract and separate the components which were consequently subjected to gravimetric analysis, titration and spectrophotometry for quantitative determination.

5 Reagents

- 5.1 Sodium hydroxide**, CAS No. 8012-01-9, with a purity of more than 95 %.
- 5.2 Sulphuric acid**, CAS No. 7664-93-9, with a purity of 95 % to 98 %, $\rho = 1,84$ g/ml.
- 5.3 Ammonium oxalate**, CAS No. 1113-38-8, with a purity of more than 99 %.
- 5.4 Anthrone**, CAS No. 90-44-8, analytical grade.
- 5.5 Grate 3 water**, in accordance with ISO 3696.
- 5.6 Acetone**, CAS No. 67-64-1, with a purity of more than 99,5 %.

- 5.7 **Ethanol anhydrous**, CAS No. 9003-99-0, with a purity of more than 99 %.
- 5.8 **Ammonium hydroxide**, CAS No. 1336-21-6, ammonia concentration 25 % to 28 %, $\rho = 0,90$ g/ml.
- 5.9 **Hydrochloric acid**, CAS No. 7647-01-0, hydrochloric acid mass fraction 36 % to 38 %, $\rho = 1,19$ g/ml.
- 5.10 **Alpha-D-Galacturonic acid monohydrate**, CAS No. 91510-62-2, with a purity of more than 97 %.
- 5.11 **Carbazole**, CAS No. 86-74-8, with a purity of more than 98 %.
- 5.12 **Anhydrous glucose**, CAS No. 50-99-7, with a purity of more than 99,5 %.
- 5.13 **Potassium sodium tartrate tetrahydrate**, CAS No. 6381-59-5, with a purity of more than 99 %.
- 5.14 **3,5-dinitrosalicylic acid**, CAS No. 609-99-4 with a purity of more than 98 %.
- 5.15 **Phenol**, CAS No. 50-95-2, with a purity of more than 99 %.
- 5.16 **Barium chloride**, CAS No. 10361-37-2, with a purity of more than 99 %.

6 Apparatus

- 6.1 **Soxhlet extraction apparatus**, set compatible with a 250 ml round bottom flask.
- 6.2 **Filter paper**, with a particle retention range of 4 μm to 7 μm and a thickness of 180 μm .
- 6.3 **Glass condenser**, spherical, 300 mm.
- 6.4 **Round bottom flasks**, 100 ml, 250 ml and 1 000 ml.
- 6.5 **Glass volumetric cylinder**, 250 ml.
- 6.6 **Filter funnel**, 100 ml, made from heat-resistant glass with glass frit between 16 μm and 40 μm (Frit type P40 specified in accordance with ISO 4793).
- 6.7 **Filter flasks**, 250 ml and 1 000 ml.
- 6.8 **Volumetric flasks**, 25 ml, 50 ml, 100 ml, 250 ml, 500 ml and 1 000 ml.
- 6.9 **Spectrophotometer**, works in the ultraviolet and visible range of 200 nm to 800 nm, compatible with 1 cm cuvette.
- 6.10 **Colorimetric tubes**, 25 ml.
- 6.11 **Electronic balance**, with a resolution of 0,01 g used for preparing test specimen.
- 6.12 **Electronic analytical balance**, with a resolution of 0,000 1 g used for preparing standard solution.

6.13 Oil bath, thermostatically adjustable from 37 °C to 150 °C.

6.14 Ventilated oven, temperature adjustable in 1 °C increments in the range of 50 °C to 150 °C.

6.15 Hot plate, temperature adjustable in 1 °C increments with maximum surface temperature of 300 °C or higher.

6.16 Glass desiccator, with a diameter of 180 mm.

6.17 Glass beakers, 100 ml, 250 ml, 500 ml and 1 000 ml.

6.18 Thermostatic water bath, thermostatically adjustable from 40 °C to 100 °C.

7 Test procedure

7.1 Preparation of standard solutions

7.1.1 Aqueous ammonium oxalate solutions

Prepare 10 g/l and 5 g/l solutions of ammonium oxalate (5.3) in water (5.5) in two 500 ml volumetric flasks (6.8), respectively.

7.1.2 Aqueous sodium hydroxide solution

Prepare 0,1 mol/l solution of sodium hydroxide (5.1) in water (5.5) in a 250 ml volumetric flask (6.8).

Prepare 0,5 mol/l solution of sodium hydroxide (5.1) in water (5.5) in a 250 ml volumetric flask (6.8).

7.1.3 Ethanol solution of Hydrochloric acid

Combine 1 000 ml of anhydrous ethanol (5.7) with 11 ml hydrochloric acid (5.9) and mix well.

7.1.4 Standard stock solution of galacturonic acid

Prepare 100 ml of 1 000 mg/l solution of galacturonic acid (5.10) in water (5.5).

7.1.5 Ethanol solution of carbazole

Prepare 50 ml of 0,15 % solution of carbazole (5.11) in ethanol (5.7).

7.1.6 Aqueous solution of Hydrochloric acid

Prepare 2 mol/l solution of hydrochloric acid (5.9) in water (5.5) in a 500 ml volumetric flask (6.8).

7.1.7 DNS chromogenic solution

Add 18,20 g potassium sodium tartrate tetrahydrate (5.13) into a 100 ml beaker (6.17) filled with 50 ml water (5.5) and heat the mixture on a hot plate (6.15) to slightly below 50 °C. To the warm solution in the beaker add 0,63 g 3,5-dinitrosalicylic acid (5.14), and 4,10 g sodium hydroxide (5.1) pre-dissolved in 15 ml to 20 ml water (5.5), and 4,16 g phenol (5.15), consecutively with stirring until complete dissolution. Let the solution cool to room temperature and transfer it to a 100 ml volumetric flask. Rinse the beaker with 5 ml water (5.5) for three times and add all rinses to the volumetric flask. Dilute with water (5.5) to the marked line.

NOTE Store this working solution in a brown bottle at room temperature for 7 days before usage. It is kept in the dark at 0 °C to 4 °C for up to 3 months.

7.1.8 Standard stock solution of glucose

Prepare 1 000 mg/l solution of glucose (5.12) in water (5.5) in a 250 ml volumetric flask (6.8).

7.1.9 Aqueous sulfuric acid solution

Slowly add 40 ml concentrated sulfuric acid (5.2) to a 100 ml beaker (6.17) filled with 26,5 ml water (5.5) with stirring. Mix well and let cool to room temperature.

7.1.10 Aqueous barium chloride solution

Prepare 0,5 mol/l solution of barium chloride (5.16) in water (5.5) in a 100 ml volumetric flask (6.8).

7.1.11 Anthrone solution in sulfuric acid

Slowly add 95 ml sulfuric acid (5.2) to a 250 ml beaker (6.17) containing 5 ml water (5.5) and chill the mixture in an ice bath. Weigh out 0,2 g anthrone (5.4) and dissolve in the cold sulfuric acid solution. Dilute the solution by slowly adding it to 20 ml water (5.5) while keep it cold using the ice bath.

NOTE This working solution is used within 1 h of preparation, kept in the refrigerator at 0 °C to 4 °C for up to 5 days.

7.2 Sampling and preparation of test specimens

7.2.1 Sampling

Sampling shall be carried out by one of the methods given in ISO 1130. Samples shall be representative of a batch.

7.2.2 Preparation of test specimen

Weigh out test specimens of about 3 g each for a batch of samples collected in 7.2.1 using an electronic balance (6.11). Cut each test specimen into small pieces with a maximum dimension less than 5 mm. Dry the test specimens at 105 °C ± 3 °C in a ventilated oven (6.14) to constant weight. Rapidly transfer the test specimens to a desiccator (6.16) and allow to cool to room temperature. Weigh the cooled test specimens with a balance (6.12) to the nearest 0,000 1 g and record as G_0 .

NOTE Constant mass is considered to be achieved when measurements made at intervals of 1 h do not show a change in mass greater than 0,02 %.

7.3 Determination of fat and wax content

7.3.1 Extraction with acetone

7.3.1.1 Take three test specimens (7.2.2) and record the original dry mass of each test specimen (G_0). Place each test specimen inside a thimble made from filter paper (6.2) and load inside the main chamber of the Soxhlet extraction apparatus (6.1). Connect the main chamber with a condenser (6.3) and place onto a 250 ml round bottom flask (6.4) filled with 100 ml acetone (5.6). Heat the acetone to reflux using an oil bath (6.13) or a water bath (6.18) set at a proper temperature so that the main chamber is filled with acetone 4 times to 6 times per hour. Allow the extraction cycle to repeat over 8 h and let it cool after the solvent is siphoned back into the flask. Take out the remnant solid with the thimble and let it air-dry in a fume hood.

7.3.1.2 Dry the remnant test specimens in a ventilated oven (6.14) at 105 °C ± 3 °C to constant mass. Rapidly transfer the test specimens to a desiccator (6.16) and allow them to cool to room temperature. Weigh the cooled test specimens to the nearest 0,000 1 g and record as G_1 .

7.3.2 Calculation

Calculate the fat and wax content (W_1), in percentage, by using [Formula \(1\)](#) and round it to one significant figure.

$$w_1 = \frac{G_0 - G_1}{G_0} \times 100 \quad (1)$$

where

w_1 is the fat and wax content, in %;

G_0 is the dry mass of the untreated test specimen, in g;

G_1 is the dry mass after extraction with acetone, in g.

Calculate the average of three test specimens and round at two significant figures. The relative standard deviation of the three measurements should not exceed 5 %. Otherwise, an additional test specimen shall be measured and the average of the three measurements shall be taken as the reported value after excluding the outlier.

7.4 Determination of pectin content

7.4.1 Development of galacturonic acid standard curve

7.4.1.1 Dispense 0 ml, 2 ml, 4 ml, 6 ml, 8 ml, and 10 ml standard galacturonic acid solution ([7.1.4](#)) into six 100 ml volumetric flasks ([6.8](#)), respectively and dilute with water ([5.5](#)) to the marked line to obtain standard solutions at concentrations of 0 mg/l, 20 mg/l, 40 mg/l, 60 mg/l, 80 mg/l, and 100 mg/l.

7.4.1.2 Place 1 ml of each standard solution in a 25 ml colorimetric tube ([6.10](#)) followed by addition of 8 ml sulfuric acid ([5.2](#)). Heat the tubes for 15 min in an oil bath ([6.13](#)) or a water bath ([6.18](#)) set at 75 °C and add 0,2 ml of carbazole solution ([7.1.5](#)) to each tube after cooling it to room temperature. Shake the tubes to allow good mixing and then keep in dark at room temperature for 30 min to allow completion of the chromogenic reaction. Measure the absorbance of the solutions at 540 nm using the spectrophotometer ([6.9](#)) and plot against the galacturonic acid concentrations to obtain the standard curve.

7.4.2 Extraction of pectin with aqueous ammonium oxalate

Take three test specimens ([7.2.2](#)) and record the original dry mass of each test specimen (G_0). Remove fat and wax by extraction with acetone ([7.3.1](#)). Place each defatted specimen in a 250 ml round bottom flask ([6.4](#)) filled with 100 ml of 10 g/l aqueous ammonium oxalate solution ([7.1.1](#)). Connect the flask with a condenser ([6.3](#)) and heat it to boil and reflux for 3 h using an oil bath ([6.13](#)) set at 110 °C. Pass the extracting solution through a filter paper ([6.2](#)) into a 500 ml beaker ([6.17](#)). The remnant solid was again extracted with 100 ml refluxed 5 g/l aqueous ammonium oxalate solution ([7.1.1](#)) for 2 h. The second extracting solution was filtered using the same filter paper ([6.2](#)) and combined with the first extracting solution in the beaker ([6.17](#)). The remnant was washed using 10 ml aqueous ammonium oxalate ([7.1.1](#)) for 3 times and the washes were combined with the extracting solutions in the beaker ([6.17](#)).

7.4.3 Precipitation of pectin

Place the beaker filled with the pectin extract ([7.4.2](#)) on a hot plate ([6.15](#)). Set the temperature so as the solution was heated to just below boiling temperature. Wait until the solution was concentrated to about 70 ml and let it cool to room temperature. Transfer the concentrated pectin extract into a 100 ml volumetric flask ([6.8](#)). Rinse the beaker with 5 ml water ([5.5](#)) for three times and add the rinses to the concentrated extract in the volumetric flask ([6.8](#)) followed by dilution to the marked line. Transfer 25 ml of this solution into a 500 ml glass beaker ([6.17](#)), slowly add 90 ml ethanol solution of hydrochloric

acid (7.1.3) with stirring. Let stand for 12 h to allow complete precipitation of the pectin. Isolate the precipitated pectin with filter paper (6.2) and wash it with 30 ml ethanol solution of hydrochloric acid (7.1.3) in 3 batches.

7.4.4 Preparation of the testing solution of pectin

Place the precipitated pectin with the filter paper (7.4.3) in a 100 ml glass beaker (6.17). Add 75 ml hot aqueous ammonium hydroxide solution, which was the mixture of 73 ml boiling water (5.5) with 1,5 ml ammonium hydroxide (5.8). Place the beaker on a hot plate (6.15) and boil for 5 min. Filter the solution into a 500 ml beaker (6.17). Boil the original filter paper with 25 ml water (5.5) for 5 min and combine the wash filtrate with the original filtrate in the beaker (6.17). Repeat the washing process for two more times and combine all filtrates. Allow the filtrate to cool to room temperature, transfer it into a 250 ml volumetric flask (6.8) and dilute with water (5.5) to the marked line.

7.4.5 Spectrophotometric testing

Spectrophotometric tests of the pectin solutions were carried out using the same colour development method described in 7.4.1 for the galacturonic acid standard curve. The absorbance of the three testing solutions were recorded and converted to the concentration of galacturonic acid according to the standard curve (7.4.1).

7.4.6 Calculation

Calculate the pectin content (W_2), in percentage, by using Formula (2) and round it to one significant figure.

$$w_2 = \frac{C_2 \times V_2 \times 4}{G_0 \times 10^3} \times 100 \quad (2)$$

where

w_2 is the pectin content (in galacturonic acid), in %;

C_2 is the concentration of galacturonic acid determined spectroscopically, in mg/l;

V_2 is the volume of the original pectin extract, in l ($V_2 = 0,25$ l);

4 is the dilution ratio;

G_0 is the dry mass of the untreated specimen, in g.

Calculate the average of three test specimens and rounded at two significant figures. The relative standard deviation of the three measurements should not exceed 5 %. Otherwise, an additional test specimen shall be measured and the average of the three measurements shall be taken as the reported value after excluding the outlier.

7.5 Determination of hemicellulose content

7.5.1 Development of glucose standard curve

7.5.1.1 Dispense 0 ml, 10 ml, 20 ml, 30 ml, 40 ml, and 50 ml standard glucose solution (7.1.8) into six 100 ml volumetric flasks (6.8) respectively and dilute with water (5.5) to the marked line to obtain standard solutions at concentrations of 0 mg/l, 100 mg/l, 200 mg/l, 300 mg/l, 400 mg/l and 500 mg/l.

7.5.1.2 Place 2 ml of each standard solution in a 25 ml colorimetric tube (6.10) followed by addition of 1,5 ml DNS standard solution (7.1.7). Heat the tubes for 5 min in an oil bath (6.13) set at $105 \text{ }^\circ\text{C} \pm 3 \text{ }^\circ\text{C}$ and then cooled to room temperature. Transfer the solutions to 25 ml volumetric flasks (6.8) and dilute

with water (5.5) to the marked line. Store the solutions for 2 h and measure the absorbance at 540 nm using the spectrophotometer (6.9) and plot against the glucose concentrations to obtain the standard curve.

7.5.2 Hydrolysis and extraction of hemicellulose by hot hydrochloric acid

Take three test specimens (7.2.2) and record the original dry mass of each test specimen (G_0). Remove fat and wax by extraction with acetone (7.3.1) and remove pectin by extraction with aqueous ammonium oxalate (7.4.2). Dry the defatted and depectinated test specimens in a ventilated oven (6.14) at $105\text{ °C} \pm 3\text{ °C}$ to constant weight. Place each test specimen in a 250 ml beaker (6.17) filled with 100 ml of 2 mol/l hydrochloric acid (7.1.6) and place the beaker on a hot plate (6.15) and boil for 1 h. Filter the solution into a 500 ml beaker (6.17). Rinse the residue on filter paper with 15 ml water (5.5) for 3 times and combine all filtrates. Allow the filtrate to cool to room temperature, transfer it into a 1 000 ml volumetric flask (6.8) and dilute with water (5.5) to the marked line.

7.5.3 Spectrophotometric testing

Place 2 ml of each diluted extract prepared in 7.5.2 in a 25 ml colorimetric tube (6.10) and adjust the pH to about 8 with addition of 0,1 mol/l sodium hydroxide solution (7.1.2) followed by addition of 1,5 ml DNS standard solution (7.1.7). Heat the tubes for 5 min in an oil bath (6.13) set at $105\text{ °C} \pm 3\text{ °C}$ and then cool it to room temperature. Transfer the solutions to 25 ml volumetric flasks (6.8) and dilute with water (5.5) to the marked line. Store the solutions for 2 h and measure their absorbance at 540 nm using the spectrophotometer (6.9) converted to the concentration of reducing sugars according to the standard curve (7.5.1).

7.5.4 Calculation

Calculate the hemicellulose content (W_3), in percentage, by using Formula (3) and round it to one significant figure.

$$w_3 = \frac{C_3 \times V_3 \times 0,9}{G_0 \times 10^3} \times 100 \quad (3)$$

where

w_3 is the hemicellulose content, in %;

C_3 is the mass concentration of reducing sugar determined spectrophotometrically, in mg/l;

V_3 is the volume of the original hemicellulose extract, in ml ($V_3 = 1$ l);

0,9 is the conversion coefficient between reducing sugar and hemicellulose;

G_0 is the dry mass of the untreated specimen, in g.

Results of three test specimens are averaged and rounded at two significant figures. The relative standard deviation of the three measurements should not exceed 5 %. Otherwise, an additional specimen shall be measured and the average of the three measurements shall be taken as the reported value after excluding the outlier.

7.6 Determination of lignin content

7.6.1 Test specimen preparation

7.6.1.1 Take three test specimens (7.2.2) and record the original dry mass of each test specimen (G_0). Remove fat and wax by extraction with acetone (7.3.1). Place each defatted test specimen in a 100 ml glass beaker (6.17) filled with 15 ml 72 % aqueous sulfuric acid solution (7.1.9). Stir the mixture until well dispersed and let stand for 10 h. Transfer the mixture into a 250 ml glass beaker (6.17) and dilute

with water (5.5) to 150 ml. Place the beaker on a hot plate (6.15) and heat to near boil for 4 h. Isolate the insoluble by vacuum filtration using a tared filter funnel (6.6). Record the dry mass of filter in the filter funnel (G_f), to the nearest 0,000 1 g. Make sure all lignin is collected by rinsing the glass beaker (6.17) three times with water (5.5) and pass the rinses through the filter funnel (6.6). Wash the filtration residue on the filter with water (5.5) until the filtrate is free of sulphate ion [No precipitation formed when dropped in the barium chloride solution (7.1.10)].

7.6.1.2 Dry the filter cake with the filter in a ventilated oven (6.14) at $105\text{ °C} \pm 3\text{ °C}$ to constant mass. Rapidly transfer the filter to a desiccator (6.16) and allow to cool to room temperature. Weigh the residue and the filter with a balance (6.12) to the nearest 0,000 1 g and record as G_4 .

NOTE Constant mass is considered to be achieved when measurements made at intervals of 1 h do not show a change in mass greater than 0,02 %.

7.6.2 Calculation

Calculate the lignin content (W_4), in percentage, by using Formula (4) and round it to one significant figure.

$$w_4 = \frac{G_4 - G_f}{G_0} \times 100 \quad (4)$$

where

w_4 is the lignin content, in %;

G_f is the dry mass of the filter, in g;

G_4 is the total dry mass of the dried filter cake and filter, in g;

G_0 is the dry mass of the untreated specimen, in g.

Calculate the average of three test specimens and rounded at two significant figures. The relative standard deviation of the three measurements should not exceed 5 %. Otherwise, an additional test specimen shall be measured and the average of the three measurements shall be taken as the reported value after excluding the outlier.

7.7 Determination of cellulose content

7.7.1 Development of glucose standard curve

7.7.1.1 Dispense 0 ml, 2 ml, 4 ml, 6 ml, 8 ml, and 10 ml standard glucose solution (7.1.8) into six 100 ml volumetric flasks (6.8) respectively and dilute with water (5.5) to the marked line to obtain standard solutions at concentrations of 0 mg/l, 20 mg/l, 40 mg/l, 60 mg/l, 80 mg/l, and 100 mg/l.

7.7.1.2 Place 1 ml of each standard glucose solution in a 25 ml colorimetric tube (6.10) and chill in an ice bath. Then add 4 ml anthrone solution (7.1.11) to each tube and keep the tubes in the ice bath for 3 min before shaking them even. Heat the tubes for 10 min in an oil bath (6.13) set at 105 °C and then cool to room temperature. Store the solutions for 2 h and measure their absorbance at 620 nm using the spectrophotometer (6.9) and plot against the glucose concentrations to obtain the standard curve.

7.7.2 Extraction and hydrolysis of cellulose

7.7.2.1 Take three test specimens (7.2.2) and record the original dry mass of each test specimen (G_0). Remove fat and wax by extraction with acetone (7.3.1). Place each defatted test specimen in a 250 ml round bottom flask (6.4) filled with 150 ml 0,5 mol/l sodium hydroxide solution (7.1.2) and reflux for

4 h. Filter out the insoluble residue, wash with 20 ml water (5.5) three times and dry the residue in a ventilated oven (6.14) at $105\text{ °C} \pm 3\text{ °C}$ to constant mass.

7.7.2.2 Place the dried filter residue (7.7.2.1) in a 100 ml glass beaker (6.17) filled with 15 ml 72 % aqueous sulfuric acid solution (7.1.9). Stir the mixture until well dispersed and let stand for 10 h. Transfer the hydrolysate into a 1 000 ml round bottom flask (6.4) filled with 300 ml water (5.5), rinse the beaker with 75 ml water (5.5) in three batches. Combined all rinses with the hydrolysate in the round bottom flask and reflux for 4 h. Filter the solution into a 1 000 ml beaker (6.17). Rinse the round bottom flask and the residue on filter paper with 15 ml water (5.5) for 5 times and combine all filtrates. Allow the filtrate to cool to room temperature, transfer it into a 1 000 ml volumetric flask (6.8) and dilute with water (5.5) to the marked line.

7.7.2.3 Transfer 2,5 ml hydrolysate (7.7.2.2) into a 100 ml volumetric flask (6.8) and dilute with water (5.5) to the marked line.

7.7.3 Spectrophotometric testing

Spectrophotometric tests of the diluted hydrolysate (7.7.2.3) were carried out using the same colour development method described in 7.7.1.2 for the glucose standard curve. The absorbance of the three testing solutions were recorded and converted to the concentration of reducing sugars according to the standard curve (7.5.1).

7.7.4 Calculation

Calculate the lignin content (W_5), in percentage, by using Formula (5) and round it to one significant figure.

$$w_5 = \frac{C_5 \times V_5 \times 0,9 \times 40}{G_0 \times 10^3} \times 100 \quad (5)$$

where

w_5 is the cellulose content, in %;

C_5 is the mass concentration of reducing sugar in the diluted hydrolysate, in mg/ml;

V_5 is the volume of the diluted hydrolysate, in l ($V_5 = 1$ l);

0,9 is the conversion coefficient between reducing sugar and cellulose;

40 is the dilution ratio;

G_0 is the dry mass of the untreated test specimen, in g.

Calculate the average of three test specimens and rounded at two significant figures. The relative standard deviation of the three measurements should not exceed 5 %. Otherwise, an additional specimen shall be measured and the average of the three measurements shall be taken as the reported value after excluding the outlier.

8 Test report

The test report shall include the following information:

- a reference to this document, (including its year of publication), i.e. ISO 5773:2023;
- date of receiving the sample and the date of test;
- description of the sample tested;

- d) the test results, including a reference to the clause which explains how the results were calculated;
- e) any deviation, by agreement or otherwise, from the procedure specified;
- f) any unusual features observed.

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