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Textiles — Determination of components in flax fibres

Textiles — Détermination des composants des fibres de lin

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Foreword

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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 38, *Textiles*, Subcommittee SC 23, *Fibres and yarns*.

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.

Textiles — Determination of components in flax fibres

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 %, ρ = 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, content 25 % to 28 %, ρ = 0,90 g/ml.

5.9 Hydrochloric acid, CAS No. 7647-01-0, content 36 % to 38 %, ρ = 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 flask, 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 flask, 250 ml and 1 000 ml.

6.8 Volumetric flask, 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, 180 mm.

6.17 Glass beaker, 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 Aqueous acetic acid solution

Prepare 1 mol/l solution of acetic acid (5.4) in water (5.5) in a 500 ml volumetric flask (6.8).

7.1.4 Ethanol solution of Hydrochloric acid

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

7.1.5 Standard stock solution of galacturonic acid

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

7.1.6 Ethanol solution of carbazole

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

7.1.7 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).

7.1.8 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.

7.1.9 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).

7.1.10 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.

7.1.11 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).

7.1.12 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 sample (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 4times to6 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 weight. 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.

$$w_1 = \frac{G_0 - G_1}{G_0} \times 100\,\% \tag{1}$$

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 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.

7.4 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 (7.1.5) 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 1 ml of each standard solution was placed in a 25 ml colorimetric tube (6.10) followed by addition of 8 ml sulfuric acid (5.2). The tubes were heated for 15 min in an oil bath (6.13) or a water bath (6.18) set at 75 °C and 0,2 ml of carbazole solution (7.1.6) was added to each tube after it was cooled to room temperature. The tubes were shaken to allow good mixing and then kept in dark at room temperature for 30 min to allow completion of the chromogenic reaction. The absorbance of the solutions at 540 nm was measured 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 sample (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 (7.1.4) 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 wish 30 ml ethanol solution of hydrochloric acid (7.1.4) 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.

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

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.