INTERNATIONAL STANDARD 2913

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION • МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ • ORGANISATION INTERNATIONALE DE NORMALISATION

Wool — Colorimetric determination of cystine plus cysteine in hydrolysates

Laine — Détermination colorimétrique de la cystine et de la cystéine dans les hydrolysats

First edition — 1975-05-15

UDC 677.31 : 677.01

Ref. No. ISO 2913-1975 (E)

Descriptors : textiles, wool, chemical analysis, determination of content, amino acids, cystine, colorimetric analysis, cysteine.

Price based on 4 pages

FOREWORD

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Draft International Standards adopted by the Technical Committees are circulated to the Member Bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 2913 was drawn up by Technical Committee ISO/TC 38, *Textiles*, and circulated to the Member Bodies in November 1972.

It has been approved by the Member Bodies of the following countries :

Australia	Germany	Portugal
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The Member Body of the following country expressed disapproval of the document on technical grounds :

France

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Wool — Colorimetric determination of cystine plus cysteine in hydrolysates

0 INTRODUCTION

Cystine is the most important amino acid in wool. Its disulphide group is very reactive and is attacked by a wide range of reagents used in wool processing. While acid treatments affect the cystine content only slightly, alkalis, oxidizing and reducing agents, steam and exposure to light and heat cause it to decrease. Since fibre damage is often associated with a decrease in cystine content, a method for its determination is useful for the control and diagnosis of degradation caused by certain agents.

The present method is based on the Folin-Shinohara method for the estimation of cystine in acid hydrolysates of proteins but it should be noted that the cystine content of intact wool is not necessarily the same as that of its hydrolysate. The method is simple to carry out, requires little special apparatus and is suitable for use in industrial laboratories.

The method consists of reductive fission of the disulphide bond of cystine with sodium disulphite, and colorimetric estimation of the cysteine formed, using dodecatungstophosphoric acid. Virgin wool contains a small amount of cysteine and this is included in the cystine plus cysteine content.

1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a method for the colorimetric determination of cystine plus cysteine in wool hydrolysates.

The method is applicable to all-wool textiles in any form, namely loose fibre, sliver, roving, yarn or cloth. Dyes when present may interfere with the colorimetric measurement. **The method is not applicable to oxidized or reduced wool** because the products of these reactions may undergo side reactions during hydrolysis, thus leading to erroneous results.

The method is therefore primarily applicable to the determination of cystine plus cysteine in washed wool, tops, yarn or cloth that have not been reduced or oxidized.

Metal-complexing agents such as EDTA and cyanide should be absent from the wool, since the reaction is copper catalysed.

2 PRINCIPLE

Hydrolysis of the wool in aqueous sulphuric acid. Reaction of the buffered hydrolysate with sodium disulphite and dodecatungstophosphoric acid, resulting in the development of a blue coloration, the intensity of which is proportional to the concentration of cystine plus cysteine in the hydrolysate. Colorimetric determination of the optical density of the blue solution and calculation of the sum of cystine plus cysteine.

NOTE — The following qualitative test, applicable to undyed wool or a hydrolysate (with dyed wool the hydrolysate must be used), can be used to determine if the wool to be tested contains a significantly higher than normal amount of cysteine.

a) Undyed wool : Immerse the sample in 1 % sodium pentacyanonitrosylferrate solution until thoroughly wetted, remove, dry between filter papers and expose to ammonia fumes. A red-violet coloration indicates cysteine residues.

b) Hydrolysate : Transfer 2 to 3 ml of hydrolysate to a test-tube, add 2 or 3 drops of sodium pentacyanonitrosylferrate solution and make alkaline with concentrated ammonia. An immediate colour change to red-violet indicates cysteine residues.

3 REAGENTS

During the analysis, use only reagents of recognized analytical reagent grade and only distilled water or water of equivalent purity.

3.1 Sulphuric acid, approximately 6 N solution.

Add 150 ml of concentrated sulphuric acid, ρ 1,84 g/ml, 95 to 98 % (m/m) solution, to 850 ml of water.

3.2 Acetate buffer, pH 5,6.

Dissolve 300 g of sodium acetate (dihydrate), 24 ml of glacial acetic acid and 1 mg of copper(II) sulphate (pentahydrate) in water and make up to 1 l.

3.3 Tungstophosphoric acid reagent.

Dissolve 200 g of sodium tungstate (dihydrate—molybdenum free) in 400 ml of water, then add 100 ml of 85 % phosphoric acid and boil gently under reflux for 1 h. Remove the condenser, add bromine or bromine water drop by drop until a bright yellow to brown colour is obtained. Boil off the excess bromine (about 15 min). Cool, filter into a 1 l measuring flask and make up to volume with water. Store the resultant clear, pale yellow-brown solution in a brown glass bottle.

3.4 Sodium disulphite solution.

Dissolve 10 g of sodium disulphite in water, make up to 100 ml and store in the dark. Do not use the reagent if more than 20 days old.

3.5 Cystine standard solution.

Dissolve 100 mg of cystine in 20 ml of sulphuric acid (3.1) in a 250 ml measuring flask and make up to volume with water.

NOTE — The cystine must be dried in a desiccator over calcium chloride, the desiccator being kept in the dark.

4 APPARATUS

4.1 Analytical balance, accurate to 0,2 mg.

4.2 Weighing bottles with ground glass stoppers.

4.3 Ventilated drying oven for drying and for hydrolysing the test specimens at 105 ± 2 °C.

4.4 Desiccator.

4.5 Measuring flasks, 100 ml and 25 ml, of high accuracy (for example conforming to ISO/R 1042, Class A or B).

4.6 Pipettes, 15 ml, 10 ml, 5 ml, 2 ml and 1 ml. The 1 ml and 5 ml pipettes used for taking aliquots of the hydrolysate and the standard cystine solution must be of the highest accuracy available (for example conforming to ISO/R 648, Class A).

4.7 Conical flasks of appropriate capacity, and **glass rods.**

4.8 Glass flasks, sintered glass filters or **funnels with filter paper** (quantitative).

4.9 Spectrophotometer, or **filter photometer** with a filter of maximum absorption at 720 nm or higher. Any instrument is suitable provided that the optical density within the range 0 to 0,7 can be read to 0,01 and estimated to the next decimal place.

5 SAMPLING AND SAMPLE PREPARATION

Take a sample representative of the bulk and sufficient to provide the following test specimens :

— two test specimens each of mass approximately 1 g for determining dry mass;

— two test specimens each of mass approximately 0,3 g for producing hydrolysates.

Remove all vegetable matter and other foreign substances from samples of loose wool, roving, etc. Dissect samples of yarn or cloth before extraction into short lengths (approximately 1 cm) of yarn. Felted material that cannot be dissected into yarn must first be cut up into small pieces. Extract the sample with dichloromethane for 1 h in a Soxhlet apparatus, at a minimum rate of 6 cycles per hour, and evaporate the dichloromethane from the cleaned sample.

6 PROCEDURE

6.1 Weighing of test specimens

Weigh successively, to an accuracy of 0,000 2 g, the test specimens described in clause 5. Use the two test specimens each of mass 1 g for determining dry mass (see 6.2), and the two specimens each of mass 0,3 g for producing hydrolysates for duplicate tests (see 6.3).

6.2 Dry mass determination

Transfer each test specimen to a weighing bottle (4.2) and dry in the drying oven (4.3) at 105 ± 2 °C. Stopper the bottle, allow it to cool in the desiccator (4.4), remove and weigh. Repeat these drying and weighing operations until constant mass has been attained.[1] Remove the test specimens, weigh the weighing bottles, and hence determine the dry mass of the test specimens. Calculate by proportion the dry mass of the hydrolysate test specimens.

6.3 Hydrolysis

Transfer one test specimen to a 100 ml conical flask (4.7), add 8 ml of the sulphuric acid solution (3.1) and place in the drying oven at 105 ± 2 °C. Shake the flask after 0,5 — 1 — 1,5 — 2 and 2,5 h. After 10 h, remove the flask, cool to room temperature, transfer quantitatively to a 100 ml measuring flask (4.5), make up to volume with distilled water and mix thoroughly. Filter at least 50 ml through a dry, sintered glass filter or through dry filter paper (4.8).

6.4 Optical density measurements

6.4.1 *General*

All optical density measurements shall be made at a wavelength between 720 and 890 nm, preferably using 10 mm cells. If a cell of a different size is used, the results shall be suitably corrected. In the following instructions and calculations it is assumed that a 10 mm cell has been used.

The optical density value must be smaller than 0,70; otherwise a cell of a smaller size or a smaller quantity of hydrolysate must be used.

1) Constant mass has been attained when the mass of a specimen, after being re-dried for at least 30 min, does not change by more than 0,000 2 g.

6.4.2 *Reference solution*

For all optical density measurements, use distilled water as the reference solution.

6.4.3 *Cysteine and adventitious reducing agents*

Transfer 5 ml of hydrolysate (6.3) to a 25 ml measuring flask (4.5), add 15 ml of buffer solution (3.2), followed by 2 ml of tungstophosphoric acid reagent (3.3). Mix thoroughly and allow to stand for 20 to 30 min. Make up to volume with distilled water, mix thoroughly and measure the optical density. Divide the value obtained by 5 and designate it A.

6.4.4 *Cystine, cysteine (twice) and adventitious reducing agents*

Transfer 1 ml of hydrolysate (6.3) to a 25 ml measuring flask (4.5), add 5 ml of buffer solution (3.2), 1 ml of sodium disulphite solution (3.4) and 2 ml of tungstophosphoric acid reagent (3.3). Mix thoroughly and allow to stand for 20 to 30 min. Make up to volume with distilled water, mix thoroughly and measure the optical density. Make this determination in duplicate and designate the average value B.

6.4.5 *Standardization*

Prepare a solution for standardizing the colorimeter as in 6.4.4, using 1 ml of cystine standard solution (3.5) in place of the hydrolysate. Make this determination in duplicate and designate the average value C. C must be within the range 0,56 to 0,70.

7 EXPRESSION OF RESULTS

Calculate the percentage, S, of cystine plus cysteine from the formula

$$S = \frac{100\,(B - A)}{25 \times C \times m}$$

where m is the dry mass, in grams, of the test specimen.

8 CONTROLS

It is recommended that each series of tests should be checked by including a control sample having a known cystine plus cysteine content. A suitable sample for this purpose is undyed worsted yarn.

9 TEST REPORT

Indicate in the test report :

a) the result obtained;

b) the individual results;

c) reference to this International Standard;

d) any departure from the test procedure, for example owing to insufficient material being available;

e) all procedural details not provided for in this International Standard and liable to have influenced the results.

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