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Textiles — Determination of index ingredient from coloured textile —

Part 8: Hibiscus

Textiles — Détermination d'indicateurs d'ingrédients de textiles colorés —

Partie 8: Hibiscus

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Foreword

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This document was prepared by Technical Committee ISO/TC 38, *Textiles*.

A list of all parts in the ISO 22195 series can be found on the ISO website.

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

There is no doubt that dyeing plays the most important role in expressing the colour of clothes. Until the invention of synthetic dyes capable of expressing diverse colours, materials obtained from nature to dye fabric have been used. Typically, colourants were obtained from plants or various materials were extracted from minerals or insects. When dyeing fabrics using materials derived from these natural substances, it becomes necessary to identify which substances the colourant was derived from. In other words, there has been a demand to confirm whether a fabric is dyed using a natural substance.

A test method is developed to identify which type of natural substances has been used.

Textiles — Determination of index ingredient from coloured textile —

Part 8: Hibiscus

1 Scope

This document specifies a test method for the determination of the index ingredient of chemicals in coloured textile with aqueous extracts from the flowers of Hibiscus.

2 Normative references

The following document is 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 3696, *Water for analytical laboratory use — Specification and test methods*

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 https://www.electropedia.org/

3.1
Hibiscus
common name for a genus of flowering plants in the *Malvaceae* family

Note 1 to entry: The flowers of Hibiscus contain anthocyanins which are textile colourant is a genus of flowering plants in the *Mallow* family, *Malvaceae*. The genus comprises several hundred species that are native to warm temperate, subtropical and tropical regions. The flowers and leaves of *Hibiscus-rosa-sinensis*, a species of tropical Hibiscus, contain anthocyanins such as delphinidin and cyanidin that gives red colour to the dyed textile.

3.2
coloured
expressing of colours to textiles by dyeing or printing

3.3
natural colourant
colourant obtained from plants, wood, rocks, soil, insects or any other thing existing on earth without any chemical reaction adopted before colouring of textiles

4 Principle

Natural colourants usually contain several chemical constituents. Depending on the type of natural colourant, each contains a distinctive chemical. This characteristic chemical remains in the fabric dyed

with natural colourant. Therefore, analysis of natural coloured fabrics by chromatography can detect characteristic chemicals depending on the kind of natural colourant.

NOTE If the index component delphinidin 3-sambubioside is detected through this test method, it cannot be said that it is necessarily stained with Hibiscus alone. However, based on this principle, applying this test method to unknown coloured fabrics or textiles is useful to provide a minimum amount of information that can be used to confirm whether the fabric is coloured using aqueous solution of Hibiscus.

5 Reagent

Unless otherwise specified, use only reagents of recognized HPLC grade.

5.1 Water, glass double distilled water or grade 2 water complying with ISO 3696.

5.2 Ethanol (CAS No. 64-17-5).

5.3 Acetonitrile (CAS No. 75-05-8).

5.4 Formic acid (CAS No. 64-18-6), volume fraction of 30 %.

5.5 Delphinidin 3-sambubioside chloride (CAS No. 53158-73-9), reference standard with percentage purity indication e.g. 90 % or more

6 Apparatus

6.1 Analytical balance, resolution at 0,001 g.

6.2 Ultrasonic water bath, to be set up at (30 ± 2) °C.

6.3 Borosilicate glass container with stopper, 50 ml.

6.4 Membrane filter, with 0,2 µm pore size.

6.5 Liquid chromatograph (LC) with mass spectroscopy (MS).

7 Procedure

7.1 Standard preparation

Stock solution of delphinidin 3-sambubioside chloride (5.5) is prepared in ethanol (5.2) containing 1 000 mg/l.

The diluted standards of delphinidin 3-sambubioside chloride (5.5) at 1 mg/l, 10 mg/l, 20 mg/l, 50 ml/l and 100 mg/l are prepared for the calibration points.

The aqueous solution of Hibiscus colourant was dissolved with ethanol (5.2).

7.2 Preparation of test specimen

Cut the test specimen into pieces of approximately 5 mm × 5 mm and approximately 1 g. Weigh it to the nearest 0,01 g, and then place it into the glass container (6.3).

Pipette 10 ml of ethanol (5.2) each into the other glass container (6.3) and pour it to cut test specimen containing glass container and place it into an ultrasonic bath (6.2) at (30 ± 2) °C for (20 ± 1) min. Afterwards, let the extract cool down to room temperature.

Filter about 1 ml of the extracted solution into a HPLC vial using disposable syringe equipped with a membrane filter (6.4). The extracted solution may be diluted so that the peak of interest in detectable in the chromatogram. The dilution factor (f) should be noted.

7.3 Analysis

The detection and qualification of delphinidin is conducted using LC-MS (6.5) with ESI mass spectrometer. The recommended chromatographic conditions are given in Annex A.

7.4 Determination and calculation

7.4.1 Determination of delphinidin 3-sambubioside

Comparison between analyses of standard and test specimen through 7.3 can show the result of existence of delphinidin 3-sambubioside in test specimen.

Detection of delphinidin 3-sambubioside can vary due to conditions of colored test specimen. In this case, the amount of test specimen and extraction solution can be modified, and concentration of extracted solution can be adopted. The modified test specimen preparation conditions should be described in test result.

7.4.2 Calibration curve

Calibration curves with standards of delphinidin 3-sambubioside at 1 mg/l, 10 mg/l, 20 mg/l, 50 ml/l, and 100 mg/l are prepared with at least 5 calibration points. The area value of the peak is used for its quantification.

NOTE Concentration ranges for the calibration standards are subject to change upon the need of each laboratory and equipment used.

For quantification, the calibration curve shall have a correlation coefficient greater than 0,995 (R^2 greater than 0,990).

7.4.3 Calculation of delphinidin 3-sambubioside

Calculate the concentration of each delphinidin 3-sambubioside in the test specimen, in µg/g from Formula (1):

$$C_s = \frac{C_1}{W} \times f \times V \quad (1)$$

where

C_S is the concentration of each delphinidin 3-sambubioside in the test specimen, in µg/g;

C_1 is the concentration of each delphinidin 3-sambubioside in the test specimen solution, in µg/ml;

W is the mass of the test specimen, in g;

f is the dilution factor;

V is the final extraction volume, in ml.

8 Test report

The test report shall include the following information:

a) a reference to this document, i.e. ISO 22195-3:2024;

b) identification of the test specimen;

c) detection result of delphinidin;

d) conditions of chromatographic analysis;

e) any deviation from the specified procedure in this document;

f) date of the test.

Annex A
(informative)

Example of test result

A.1 Analysis of Hibiscus colourant

A.1.1 General

The powdered Hibiscus colourant was dissolved with ethanol tested according to 7.3. The result chromatogram is shown Figure A.1.

A.1.2 Chromatographic conditions for the LC-MS

As the instrumental equipment of the laboratories may vary, no general applicable parameters can be provided for chromatographic analyses.

— Mobile Phase: (phase A) water (phase B) acetonitrile, both in 0,1 % formic acid

— Column: Phenomenex Omega 1,6 µm Pola C18 (150 mm × 2,1 mm)

— Column Oven: 35 °C

— Flow rate: 0,3 ml/min

— Injection: 3 µl

— Ionization: ESI-, ESI+

— Data acquisition: scan 200 *m/z* to 1 500 *m/z*

— Nebulizer gas flow: 1,5 l/min

— Interface temperature: 250 °C

— Interface voltage: 3 500 V