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**Textiles — Determination of index  
ingredient from coloured textile —**

**Part 6:  
Punica granatum**

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

*Partie 6: Grenadier*

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## 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](http://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](http://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](http://www.iso.org/iso/foreword.html).

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](http://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 today, humankind used materials obtained from nature to dye fabric. Typically, colourants were obtained from plants or various materials were extracted from minerals or insects. Dyeing fabrics using materials derived from these natural substances made it necessary to identify which substances the colourant was derived from. In other words, there has been a demand to confirm whether a fabric has been dyed with a natural substance.

There are several natural dyes raw material which give similar colour tone, they have different colouring molecule and the precise colorant. But each has different environmental profile which decided Environment impact of dyestuff. Textile dyed with natural dyes is claimed for environmental benefit mainly. Identification of dye helps in knowing and verifying the claims, that will help environment to get benefit exactly in the way it is claimed with textile.

This leads to the development of a test method to determine the type of natural substances used.

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# Textiles — Determination of index ingredient from coloured textile —

## Part 6: Punica granatum

### 1 Scope

This document specifies a test method which identifies the index ingredient chemical included in coloured fabric with punica. Punica can be applied to both natural fibre and man-made fibre.

### 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 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 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

##### **punica**

fruit-bearing deciduous shrub in the family

Note 1 to entry: *Punica granatum*, pomegranate is a common name for punica which is a plant *Punica granatum*. The extract of fruit rind, punica is used a natural dye for colouring textile.

#### 3.2

##### **coloured**

expressing of colours to textiles by dyeing or printing

#### 3.3

##### **natural colourant**

materials 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 are usually composed of several phyto chemicals. Depending on the type of natural colourant, each contains distinctive chemical constituents. 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 On the other hand, if the index component punicalagin is detected through this test method, it cannot be said that it is necessarily stained with Punicalagin. 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 punica dye.

## 5 Reagents

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

5.1 **Water**, distilled or grade 3 according to ISO 3696.

5.2 **Acetonitrile**, HPLC grade.

5.3 **Trifluoroacetic acid**, HPLC grade.

5.4 **Formic acid**, with a volume fraction of 30 %.

5.5 **Punicalagin**, a 99 %.

## 6 Apparatus

6.1 **Analytical balance**, with resolution at 0,001 g.

6.2 **Ultrasonic water bath**, to be set up at  $(30 \pm 2)^\circ\text{C}$ .

6.3 **Silica borate glass container**, with a nominal volume of 50 ml.

6.4 **Membrane filter**, with 0,45  $\mu\text{m}$  pore size.

6.5 **Pipette**, with a nominal volume of 20 ml.

6.6 **Disposable syringe**, with a nominal volume of 2 ml.

6.7 **High performance liquid chromatograph (HPLC) with photo diode array (PDA) detector**.

## 7 Procedure

### 7.1 Standard preparation

Stock solution of punicalagin (5.5) is prepared in trifluoroacetic acid (5.3) [a volume fraction of 20 % in water (5.1)] containing 1 000 mg/l.

### 7.2 Preparation of specimen

Cut the sample into pieces of approximately (5 mm × 5 mm). Prepared approximately 2 g of the cut sample, weigh it to nearest 0,01 g (6.1), and then place it into the glass container (6.3).

Pipette 20 ml of Trifluoroacetic acid (5.3) into the other glass container (6.3) and it poured to cut sample containing glass container. Place the sample contained glass container into an ultrasonic bath at  $(30 \pm 2)^\circ\text{C}$  for  $(20 \pm 1)$  min. Afterwards, let the extract cool down to room temperature.

Filter (6.4) about 1 ml of the extracted solution into a HPLC vial using disposable syringe (6.6) equipped with a membrane filter (6.4).

### 7.3 Analysis

The detection and qualification of punicalagin is conducted using HPLC-PDA (6.7). The recommendable chromatographic conditions are given in Annex A. Confirm that the retention time and the maximum absorption wavelength of the catechin standard match, or the spectrum matches.

### 7.4 Qualification of punicalagin

Comparison between analyses of standard and sample through 7.3 can show the result of existence of punicalagin in sample.

NOTE Detection of punicalagin can be variable due to conditions of coloured sample. In this case, the amount of specimen and extraction solution can be modified and concentration of extracted solution can be adopted.

## 8 Test report

The test report shall include the following information:

- a) a reference to this document, i.e. ISO 22195-6:2021;
- b) identification of the sample;
- c) conditions of chromatographic analysis;
- d) test result;
- e) any deviation from the specified procedure in this document;
- f) the date of the test.