TECHNICAL SPECIFICATION

# ISO/TS 3632-2

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# Saffron (*Crocus sativus* L.) —

## Part 2: Test methods

*Safran (*Crocus sativus *L.) —*

*Partie 2: Méthodes d'essai*



Reference number
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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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# Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of normative document:

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An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

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.

ISO/TS 3632-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 7, *Spices and condiments*.

This first edition of ISO/TS 3632-2 cancels and replaces ISO 3632-2:1993, which has been technically revised.

ISO/TS 3632 consists of the following parts, under the general title *Saffron (*Crocus sativus *L.)*:

— *Part 1: Specification*

— *Part 2: Test methods*

# Saffron (*Crocus sativus* L.) —

# Part 2:
# Test methods

## 1 Scope

This part of ISO/TS 3632 specifies methods for the analysis of saffron obtained from *Crocus sativus* L. flowers.

It is applicable to saffron in both of the following forms:

— whole and cut filaments as a loose, supple, elastic and hygroscopic mass of filaments;

— powder.

NOTE The specifications for saffron are given in ISO/TS 3632-1.



## 2 Normative references

The following referenced documents are indispensable for the application 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 928, *Spices and condiments — Determination of total ash*

ISO 930, *Spices and condiments — Determination of acid-insoluble ash*

ISO 941, *Spices and condiments — Determination of cold water-soluble extract*

ISO/TS 3632-1, *Saffron (*Crocus sativus *L.) — Part 1: Specification*

## 3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/TS 3632-1 and the following apply.

**3.1**
**moisture and volatile matter content**
loss of mass determined under the conditions specified in this part of ISO/TS 3632

NOTE Moisture and volatile matter content is expressed as a percentage by mass of the sample.

**3.2**
**colouring strength**
$E_{1cm}^{1\%}$
absorbency of the maximum wavelength (about 440 nm) of crocines for a 1 % solution of the test sample for a 1 cm cell

NOTE Colouring strength is mainly due to the content of crocines.

**3.3**
**UV-Vis profile**
spectrum of the aqueous extract of saffron between 200 nm and 700 nm

NOTE An example is given for information in Figure C.1.

**3.4**
**detection limit**
smallest concentration or content of the analyte that can be detected, but not quantified, under the specified test conditions for a method

## 4 Tests and sample sizes

### 4.1 Minimum mass of the test sample

Considering the high cost of saffron, the mass of sample received in the laboratories for carrying out the tests is often limited. The minimum mass of the laboratory sample shall be 14 g (7 g × 2) in order to carry out the standard analyses in duplicate.

It is recommended that larger quantities of sample be placed at the disposal of the laboratories in case of dispute.

Since the mass of the test portion is low, it is advisable that it be taken from a homogenized sample.



### 4.2 Tests required and sample sizes

See Table 1 for saffron in filaments and cut filaments, and Table 2 for saffron in powder.

**Table 1 — Saffron in filaments and cut filaments**

| Analysis order | Procedure (sample: 7 g × 2 = 14 g) | Test sample g | Comments | Corresponding clause |
|---|---|---|---|---|
| 1 | Identification test | 5 | Non-destructive test<br>Reject sample if vegetable matter is found other than from *Crocus sativus* L. | Clause 5 |
| 2 | Determination of floral waste content | 3 | Non-destructive test | Clause 8 |
| 3 | Determination of foreign matter | 3 | Sample is reconstituted after re-incorporating floral waste | Clause 9 |
| 4 | Regrouping and mixing of all elements separated in tests. | 5 | Return to the original test sample of 5 g | Clauses 5, 8 and 9 |
| 5 | Microscopic examination | 0,05 | | Clause 6 |
| 6 | Determination of moisture and volatile matter content | 2,5 | Keep the sample for determination of total ash and acid-insoluble ash | Clause 7 |
| 7 | Determination of extract soluble in cold water | 2 | | Clause 11 |
| 8 | Determination of total ash | 2 (approx.) | Sampling remaining after test 6 | Clause 12 |
| 9 | Determination of acid-insoluble ash | | Sampling remaining after test 8 | Clause 13 |
| 10 | Crushing and sieving | 5 | Carry out the sieving in accordance with Clause 10 to obtain a powder of which 95 % passes through a 500 µm sieve. Re-incorporate whatever remains on the sieve in the receptacle of the sieve | Clause 10 |
| 11 | Determination of main characteristics | 0,5 | | Clause 14 |
| 12 | Thin-layer chromatography: identification of saffron pigments | 0,05 | | Clause 15 |
| 13 | Thin-layer chromatography: identification of artificial colourants | 0,5 | HPLC (test 14) may alternatively, or additionally, be performed. In the latter case, use the extract for both methods | Clause 16 |
| 14 | High-performance liquid chromatography: identification of artificial colourants | 0,5 | TLC (test 13) may alternatively, or additionally, be performed. In the latter case, use the extract for both methods | Clause 17 |
| NOTE There will remain 1,40 g of sample which can be used for further tests or for repeating certain analyses if necessary. | | | | |

**Table 2 — Saffron in powder form**

| Analysis order | Procedure (sample: 7 g × 2 = 14 g) | Test sample g | Comments | Corresponding clause |
|---|---|---|---|---|
| 1 | Identification test | 0,5 | Do not continue with the analysis if the colorimetric analysis is not correct | Clause 5 |
| 2 | Microscopic examination | 0,05 | | Clause 6 |
| 3 | Determination of moisture and volatile matter content | 2,5 | Keep the sample for determination of total ash and acid-insoluble ash | Clause 7 |
| 4 | Determination of extract soluble in cold water | 2 | | Clause 11 |
| 5 | Determination of total ash | 2 (approx.) | Sampling remaining after test 3 | Clause 12 |
| 6 | Determination of acid-insoluble ash | | Sampling remaining after test 5 | Clause 13 |
| 7 | Crushing and sieving | 4,5 | Verify that 95 % of the powder passes through a 500 µm sieve. Re-incorporate whatever remains on the sieve in the receptacle of the sieve | Clause 10 |
| 8 | Determination of main characteristics | 0,5 | | Clause 14 |
| 9 | Thin-layer chromatography: identification of saffron pigments | 0,05 | | Clause 15 |
| 10 | Thin-layer chromatography: identification of artificial colourants | 0,5 | HPLC (test 11) may alternatively, or additionally, be performed. In the latter case, use the extract for both methods | Clause 16 |
| 11 | High-performance liquid chromatography: identification of artificial colourants | 0,5 | TLC (test 10) may alternatively, or additionally, be performed. In the latter case, use the extract for both methods | Clause 17 |
| NOTE There will remain 0,9 g of sample which can be used for further tests or for repeating certain analysis if necessary. | | | | |



## 5 Identification test

### 5.1 General

This preliminary test may make the subsequent analyses unnecessary if it shows that the saffron is not pure.

### 5.2 Saffron in filaments and cut filaments

#### 5.2.1 Principle

The saffron is examined visually with a magnifying glass.

#### 5.2.2 Apparatus

**5.2.2.1** **Magnifying glass**, with a magnification of ×10 max.

**5.2.2.2** **Watch glass**, of suitable size.

#### 5.2.3 Procedure

Spread out the test sample of saffron in filaments and cut filaments on the watch glass (5.2.2.2) and examine it with the magnifying glass (5.2.2.1).

#### 5.2.4 Interpretation of results

All the filaments shall belong to the plant *Crocus sativus* L.

Reject the sample if vegetable matter other than that belonging to *Crocus sativus* L. is found.

### 5.3 Saffron in powder form

#### 5.3.1 Principle

A colorimetric reaction is used.

#### 5.3.2 Reagents

Use only reagents of recognized analytical grade, and distilled or demineralized water or water of equivalent quality.

**5.3.2.1** **Sulfuric acid**, of density 1,19 g/l.

**5.3.2.2** **Diphenylamine**, not producing any coloured reaction with the sulfuric acid.

**5.3.2.3** **Diphenylamine solution**, prepared as follows.

Add 0,1 g of diphenylamine (5.3.2.2) to 20 ml of sulfuric acid (5.3.2.1) and 4 ml of water.



#### 5.3.3 Apparatus

**5.3.3.1** **Porcelain dish**, with flat bottom.

#### 5.3.4 Procedure

Take a 0,5 g sample of saffron (see Table 2).

Place this test portion in the porcelain dish (5.3.3.1) containing the diphenylamine solution (5.3.2.3).

#### 5.3.5 Interpretation of results

Pure saffron immediately turns to a blue colour which rapidly turns reddish brown.

In the presence of nitrates, the blue colour persists.

## 6 Microscopic examination of saffron

### 6.1 General

The method is applicable to the examination of saffron in powder and filament form in order to determine whether the sample consists exclusively of stigmata belonging to *Crocus sativus* L. and to highlight any floral waste and foreign matter.

## 6.2 Principle

The identity of saffron in powder and crushed filament form is verified. Foreign matter and floral waste, if any, are identified by the observation of anatomical elements by using microscopy under the conditions described in 6.5. The percentages relating to the observed elements may be determined if required (see Annexes B and C).

## 6.3 Reagents

Unless otherwise indicated, use only reagents of recognized analytical grade, and distilled or demineralized water or water of equivalent purity.

**6.3.1 Iodine/iodide solution**, aqueous solution of iodine in potassium iodide.

In a 100 ml one-mark volumetric flask, equipped with a glass stopper, add 2 g of iodine, 4 g of potassium iodide and about 10 ml of water. Leave until completely dissolved, then make up to the mark with water. Stopper the flask.

**6.3.2 Illuminating solution**, either sodium hydroxide or potassium hydroxide with 5 g/100 ml of water, or chloral hydrate with 80 g/100 ml of water; dissolve when hot.

## 6.4 Apparatus

Usual apparatus used for microscopic examinations, such as slides, cover-glasses, scalpel, lancet needles, and the following.



**6.4.1 Volumetric flask**, of capacity 100 ml.

**6.4.2 Syringe**, permitting delivery of 50 µl, graduated in microlitres.



**6.4.3 Microscope**, permitting observation with a magnification of ×100 and ×400, equipped with a device permitting observation in polarized light (optional).

## 6.5 Procedure

### 6.5.1 Test portion

For each slide (6.5.2 to 6.5.4), take a test portion of the order of 0,001 g to 0,002 g of saffron in powder (see 10.3) or crushed filament (see 10.2) form.

### 6.5.2 Preparation for observation in water

Prepare two slides as follows.

Deposit 50 µl of water on a slide. Using the tip of a scalpel or lancet needle, take the test portion (6.5.1), mix it with the water and wait for at least 5 min until the powder is well wetted before covering with a cover slide.

### 6.5.3 Preparation for observation in an aqueous solution of sodium hydroxide, potassium hydroxide or chloral hydrate

Prepare two slides as indicated in 6.5.2 but replacing water with the sodium hydroxide, potassium hydroxide or chloral hydrate aqueous solutions (6.3.2).

Wait for a few minutes for the medium to illuminate and observe for 10 min after adding the illuminating solution in order to avoid altering the cellular elements and to ensure they can be identified.

NOTE This observation enables illumination of the preparations by destroying totally or partially the major part of the cellular contents. The cellular elements are also made clearer and easier to observe, particularly the sclerous elements, vessels, fibres and epidermis.

### 6.5.4 Preparation for observation in aqueous iodine/iodide solution

Prepare a slide as indicated in 6.5.2 but replace the water with iodine/iodide solution (6.3.1).

NOTE This observation makes visible the starch grains which are stained blackish blue or blackish violet.

### 6.5.5 Observation, identification and counting

Place each slide prepared according to 6.5.2 to 6.5.4 under the microscope (6.4.3). Set the magnification at ×100. Identify and count the elements observed with a magnification of ×400 (see 6.7).

NOTE The anatomical structures and exogenous elements are identified and counted for each slide on an observation of 10 fields.

If the microscope used (see 6.4.3) is equipped with a device permitting the observation in polarized light, one of the two slides prepared in 6.5.2 shall be observed in polarized light as indicated in 6.5.5.

Figure 1 shows an example which summarizes all operations permitting counting.

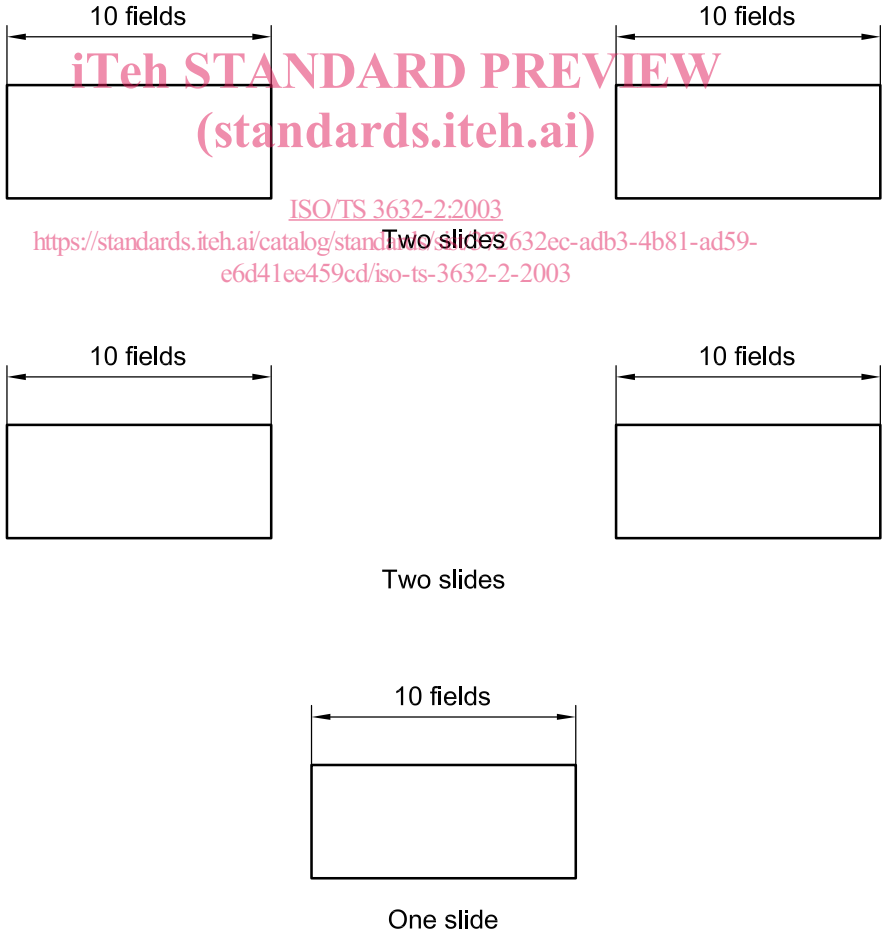




**Figure 1 — Example of counting procedure**

### 6.6 Expression of results

An example of how to express the results is given in Annex A.

### 6.7 Microscopic examination

See reference photographs given for information in Annex B.

During the examination, the following elements can be observed:

— fragments of the top extremity of the stigmata with large hair-like elongated papillae, after crushing the isolated papillae (Figure B.1);

— epidermic debris of the stigmata which are characterized by small intussusceptions of the membrane (Figure B.2);

— debris of the epidermis of the style, characterized by a sinuous wall (Figure B.3);

— round pollen grains with a diameter of between 80 µm and 100 µm, with a smooth cell wall and finely granular exine (Figure B.4);

— fragments of conductor elements made up of spiralled vessels (Figure B.5);

— fragments of stamens (Figure B.6);

— grains of starch (Figure B.7);

— inorganic matter (Figure B.8);

— fragments of straw (Figure B.9);

— cells whose contents remain coloured despite the illuminating solution (Figure B.10).



### 6.8 Interpretation of microscopic observations

Evaluation of the relative percentage of each structure from the count table permits a check that the crushed saffron is mainly made up of fragments of stigmata to which fragments of styles and grains of pollen may be associated.

The content of floral waste shall be low and the content of foreign elements shall be practically non-existent.

NOTE The crushed saffron does not have sclerous cells, fibres, covert hair or starch grains. The contents of the cells dissolve in water to give an orange-yellow colour.

## 7 Determination of moisture and volatile matter content

### 7.1 General

This method is applicable to saffron in filaments and cut filaments or in powder form.

NOTE The method of determination of the moisture content of spices and condiments described in ISO 939 is not applicable in the case of saffron because it requires the use of too large a test portion.

### 7.2 Principle

The sample is oven dried at 103 °C ± 2 °C for 16 h.