BS ISO 3632-2:2010

BSI Standards Publication

Spices — Saffron (*Crocus sativus* L.)

Part 2: Test methods

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National foreword

This British Standard is the UK implementation of ISO 3632-2:2010. It supersedes DD ISO/TS 3632-2:2003 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/7, Spices and condiments.

A list of organizations represented on this committee can be obtained on request to its secretary.

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Part 2:
Test methods

*Épices — Safran (*Crocus sativus *L.) —*

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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.

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.

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

This second edition of ISO 3632-2 cancels and replaces ISO/TS 3632-2:2003, which has been technically revised.

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

— *Part 1: Specification*

— *Part 2: Test methods*

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INTERNATIONAL STANDARD ISO 3632-2:2010(E)

Spices — Saffron (*Crocus sativus* L.) —

Part 2:
Test methods

1 Scope

This part of ISO 3632 specifies test methods for dried saffron obtained from the *Crocus sativus* L. flower.

It is applicable to saffron:

a) filaments and cut filaments;

b) powder.

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 948, *Spices and condiments — Sampling*

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

3 Terms and definitions

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

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

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

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3.2
colouring strength

$A_{1\,\mathrm{cm}}^{1\,\%}$, 440 nm

absorbance at the maximum wavelength (about 440 nm) of crocins for a 1 g/100 ml solution of test sample using a 1 cm quartz cell

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

3.3
UV-vis profile of saffron extract
spectrum obtained between 200 nm and 700 nm

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

3.4
limit of detection
LOD
minimum amount or concentration of the analyte in a test sample which can be detected reliably, but not necessarily quantified, as demonstrated by a collaborative trial or other appropriate validation

3.5
minimum required performance limit
MRPL
minimum content of an analyte in a sample, which at least has to be detected and confirmed

4 Tests and sample sizes

4.1 Minimum mass of the laboratory sample

Sampling methods for spices and condiments are specified in ISO 948.

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 23 g (11,5 g × 2) for saffron filaments and cut filaments and 13,5 g (6,75 g × 2) for saffron powder 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 test sample sizes

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

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Table 1 — Saffron in filaments and cut filaments forms

Analysis step	Procedure (laboratory sample: 11,5 g × 2 = 23 g)	Test sample g	Comments	Corresponding clause
1	Identification test	5	New test sample Non-destructive test	5
2	Microscopic examination	0,05	Test sample from step 1	6
3	Determination of floral waste content	3	Test sample from step 1 Non-destructive test	8
4	Determination of foreign matter	3	Test sample from step 3 Test sample is reconstituted after reincorporating floral waste	9
5	Determination of extract soluble in cold water	2	Test sample from step 4	11
6	Determination of moisture and volatile matter content	2,5	New test sample Keep the test sample for determination of total ash and acid-insoluble ash	7
7	Determination of total ash	2	Remains of the test sample from step 6	12
8	Determination of acid-insoluble ash	—	Test sample produced after step 7	13
9	Crushing and sieving	4	New test sample Carry out the sieving in accordance with Clause 10 to obtain a powder of which 95 % mass fraction passes through a 500 µm sieve. Reincorporate whatever remains on the sieve in the receptacle of the sieve	10
10	Determination of main characteristics	0,5	Test sample from step 9, after sieving	14
11	Thin-layer chromatography (TLC): identification of artificial colorants	0,5	Test sample from step 9, before sieving HPLC (step 12) may alternatively or additionally be performed. In the latter case, use the extract for both methods	15
12	High performance liquid chromatography (HPLC): identification of artificial colorants	0,5	Test sample from step 9, before sieving TLC (step 11) may alternatively or additionally be performed. In the latter case, use the extract for both methods	16
NOTE	There remain 4,50 g laboratory sample which can be used for further tests or to repeat certain analyses if necessary.			

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Table 2 — Saffron in powder form

Analysis step	Procedure (laboratory sample: 6,75 g × 2 = 13,5 g)	Test sample g	Comments	Corresponding clause
1	Identification test	0,2	New test sample Do not continue with the analysis if the colorimetric analysis is not correct	5
2	Microscopic examination	0,05	New test sample	6
3	Determination of moisture and volatile matter content	2,5	New test sample Keep the test sample for determination of total ash and acid-insoluble ash	7
4	Determination of total ash	2	Remains of the test sample from step 3	12
5	Determination of acid-insoluble ash	—	Remains of the test sample from step 4	13
6	Crushing and sieving	4	New test sample Verify that 95 % mass fraction of the powder passes through a 500 µm sieve. Reincorporate whatever remains on the sieve in the receptacle of the sieve	10
7	Determination of extract soluble in cold water	2	Test sample from step 6	11
8	Determination of main characteristics	0,5	Test sample from step 6, after sieving	14
9	Thin-layer chromatography (TLC): identification of artificial colorants	0,5	Test sample from step 6, before sieving HPLC (10) may alternatively or additionally be performed. In the latter case, use the extract for both methods	15
10	High performance liquid chromatography (HPLC): identification of artificial colorants	0,5	Test sample from step 6, before sieving TLC (9) may alternatively or additionally be performed. In the latter case, use the extract for both methods	16
NOTE	There remain 1 g laboratory sample which can be used for further tests or to repeat certain analyses 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 times max.

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

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5.2.3 Procedure

Spread out the test sample of saffron in filaments and cut filaments (Table 1) 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

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent quality.

5.3.2.1 **Sulfuric acid**, mass concentration 1,19 g/l.

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

The diphenylamine shall not produce any coloured reaction with the sulfuric acid.

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

5.3.4 Procedure

Use the 0,2 g test sample of saffron (see Table 2) as test portion.

Gradually add the test portion to the porcelain dish (5.3.3) containing the diphenylamine solution (5.3.2.2).

5.3.5 Interpretation of results

Pure saffron immediately produces 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, filaments, and cut filament forms in order to determine whether the sample consists exclusively of stigmas 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 A and B).

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6.3 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

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

In a 100 ml one-mark volumetric flask (6.4.5), equipped with a glass stopper, add 2 g iodine, 4 g potassium iodide, and about 10 ml 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 at a mass concentration of 5 g/100 ml water or chloral hydrate with 80 g/100 ml water; dissolve when hot.

6.4 Apparatus

Usual laboratory equipment and in particular the following.

6.4.1 Slides.

6.4.2 Cover-glasses.

6.4.3 Scalpel.

6.4.4 Lancet needles.

6.4.5 One-mark volumetric flask, capacity 100 ml, ISO 1042[4] class A.

6.4.6 Syringe, graduated in microlitres, capable of delivering volumes of 50 µl.

6.4.7 Microscope, permitting observation with a magnification of 100 times and 400 times, optionally equipped with a device permitting observation in polarized light.

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 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 to ensure that the powder is adequately wet 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 replace 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 ensuring 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.

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6.5.4 Preparation for observation in aqueous iodine in iodide solution

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

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

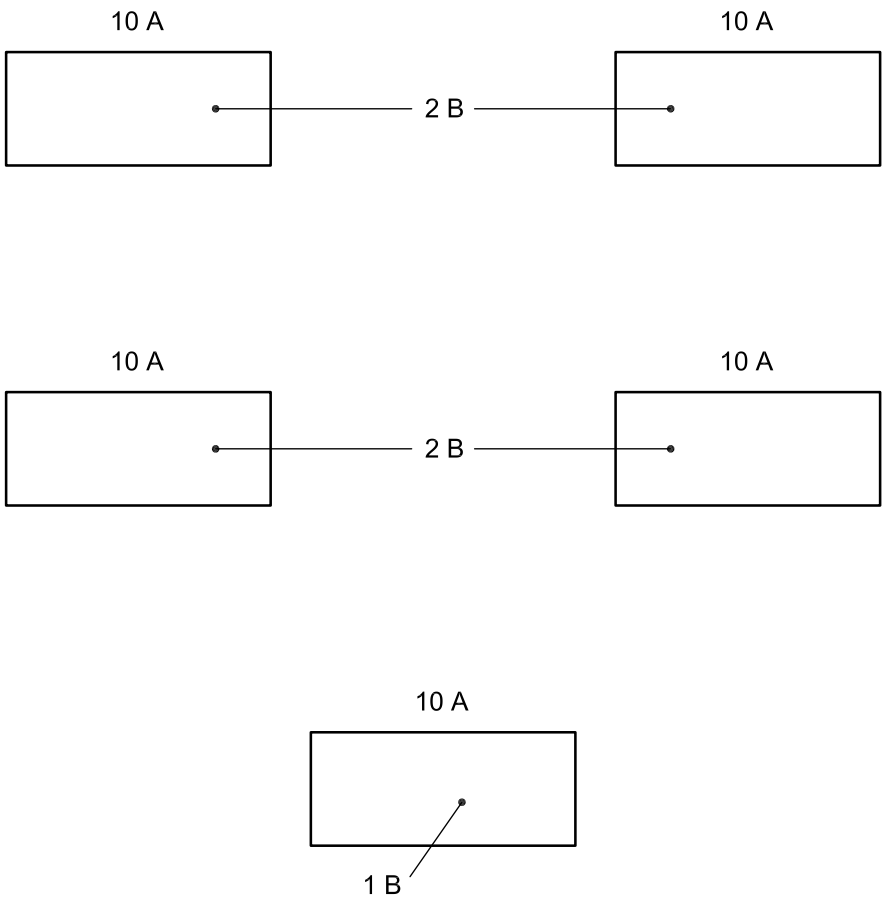
6.5.5 Observation, identification, and counting

Place each slide, prepared according to 6.5.2 to 6.5.4, under the microscope (6.4.7). Set the magnification at 100 times. Identify and count the elements observed with a magnification of 400 times (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 (6.4.7) is equipped with a device permitting observation in polarized light, one of the two slides prepared in 6.5.2 should be so observed.

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



Key

A field

B slide

Figure 1 — Example of counting procedure

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6.6 Expression of results

An example is given for information 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:

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

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

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

d) 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);

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

f) fragments of stamens (Figure B.6);

g) grains of starch (Figure B.7);

h) inorganic matter (Figure B.8);

i) fragments of straw (Figure B.9);

j) cells the content of which remain coloured despite the illuminating solution (Figure B.10).

6.8 Interpretation of microscopic observations

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

The observation should be reported in accordance with Tables A.1 and A.2.

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, cut filaments and in powder forms.

NOTE The method of determination of the moisture content of spices and condiments described in ISO 939[3] 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.

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