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Saffron (*Crocus sativus* Linnaeus) —

Part 2: Test methods

Safran (*Crocus sativus* Linnaeus) —
Partie 2: Méthodes d'essai



Reference number
ISO 3632-2:1993(E)

Foreword

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International Standard ISO 3632-2 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 7, *Spices and condiments*.

This first edition of ISO 3632-2, together with ISO 3632-1 cancels and replaces ISO 3632:1980, of which they constitute a technical revision.

ISO 3632 consists of the following parts, under the general title *Saffron* (*Crocus sativus* Linnaeus):

- Part 1: *Specification*
- Part 2: *Test methods*

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Introduction

Since saffron is a costly spice, the general methods of testing spices are not always suitable because they require the use of large test portions. This is why it was decided to include in this part of ISO 3632 the test methods specific to saffron, when it is not possible to use the general standards.

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Saffron (*Crocus sativus* Linnaeus) —

Part 2: Test methods

1 Scope

This part of ISO 3632 describes methods suitable for testing the spice saffron, which is obtained from the flowers of the saffron crocus (*Crocus sativus* Linnaeus).

It is applicable to the testing of saffron in either of the following forms:

- in whole filaments as a loose, supple, elastic and hygroscopic mass of filaments, or
- in powder form.

NOTE 1 Specifications for saffron are given in ISO 3632-1.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 3632. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this part of ISO 3632 are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 928:1980, *Spices and condiments — Determination of total ash*.

ISO 930:1980, *Spices and condiments — Determination of acid-insoluble ash*.

ISO 3632-1:1993, *Saffron (Crocus sativus Linnaeus) — Part 1: Specification*.

3 Definitions

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

3.1 moisture and volatile matter content: Loss of mass determined under the conditions described. It is expressed as a percentage by mass of the sample.

3.2 colouring strength: Mainly due to its crocine content, it is defined by measurement of the optical density at the maximum, about 440 nm.

3.3 bitterness: Mainly due to its picrocrocine content, it is defined by measurement of the optical density at the maximum, about 257 nm.

3.4 flavour: Mainly due to its safranal content, it is defined by measurement of the optical density at the maximum, about 330 nm.

4 Preparation of test sample and order of tests

4.1 Minimum mass of test sample

IMPORTANT — In view of 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 10 g (5 g × 2) so that it is possible to carry out all the usual analyses in duplicate.

Larger quantities of sample shall be placed at the disposal of the laboratories in case of any dispute, or if additional tests are required (e.g. nitrogen, crude fibre).

4.2 Procedure

4.2.1 Saffron in filaments

Carry out, **in the order indicated**, the tests and analyses according to the scheme given in table 1.

4.2.2 Saffron in powder form

Carry out, **in the order indicated**, the tests and analyses according to the scheme given in table 2.

5 Identification test

5.1 General

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

5.2 Saffron in filaments

5.2.1 Principle

Visual examination with a magnifying glass.

5.2.2 Apparatus

5.2.2.1 Magnifying glass, with a magnification of $\times 10$ max.

Table 1 — Saffron in filaments: Order of test procedures

Order	Test procedure (sample: 5 g $\times$ 2 = 10 g)	Test sample g	Comments
1	Identification test (clause 5)	5	Non-destructive test Reject sample if vegetable matter is found other than from <i>Crocus sativus</i> Linnaeus
2	Determination of floral waste content (clause 6)	3	Non-destructive test
3	Determination of extraneous matter (clause 7)	3	Sample is reconstituted after reincorporation of floral wastes
4	Regrouping and mixing of all the elements separated in tests (clauses 5 to 7)	5	Return to the original test sample of 5 g
5	Separation of test sample into sample A (3 g) and sample B (2 g)		
	Sample A (3 g)		
6A	Determination of moisture and volatile matter content (clause 9)	2,5	Keep the sample for determination of total ash and acid-insoluble ash
7A	Determination of total ash (clause 10)	2 (approx.)	Sample remaining after 6A
8A	Determination of acid-insoluble ash (clause 11)		Sample remaining after 7A
	Sample B (2 g)		
6B	Crushing and sieving (clause 12)	2	Carry out the crushing in accordance with clause 12 to obtain a powder of which 95 % passes through a 500 μ m sieve
7B	Determination of main characteristics (clause 13)	0,5	
8B	Thin-layer chromatography (clause 14)	0,05	
NOTE — There will remain 0,5 g of sample A and 1,45 g of sample B which can be used for further tests or for repeating certain analyses if necessary.			

Table 2 — Saffron in powder form: Order of test procedures

Order	Test procedure (sample: 5 g × 2 = 10 g)	Test sample g	Comments
1	Identification test (clause 5)	0,5	Do not continue with the analysis if the colorimetric analysis is not correct
2	Microscopic examination (clause 8)	0,01 to 0,02	
3	Separation of remaining test sample (4,48 g) into sample A (2,5 g) and sample B (1,98 g)		
	Sample A (2,5 g)		
4A	Determination of moisture and volatile matter content (clause 9)	2,5	Keep the sample for determination of total ash and acid-insoluble ash
5A	Determination of total ash (clause 10)	2 (approx.)	Sample remaining after 4A
6A	Determination of acid-insoluble ash (clause 11)		Sample remaining after 5A
	Sample B (1,98 g)		
4B	Sieving Crushing, if powder is > 500 µm (clause 12)	1,98	Verify that 95 % of the powder passes through a 500 µm sieve
5B	Determination of main characteristics (clause 13)	0,5	
6B	Thin-layer chromatography (clause 14)	0,05	
NOTE — There will remain 0,5 g of sample A and 1,43 g of sample B which can be used for further tests or for repeating certain analyses if necessary.			

5.2.3 Procedure

Spread out the test sample of saffron in filaments 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* Linnaeus.

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

5.3 Saffron in powder form**5.3.1 Principle**

Use of a colorimetric reaction.

5.3.2 Reagents

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

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.

5.4 Procedure

Take from sample B (see table 2) 0,5 g of saffron.

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

5.4.1 Interpretation of results

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

The blue colour shall persist in the presence of nitrates.

6 Determination of floral waste content of saffron in filaments

6.1 Principle

Physical separation of the floral waste present in a test portion and weighing.

6.2 Apparatus

6.2.1 Watch glass.

6.2.2 Small laboratory tongs.

6.2.3 Analytical balance, accurate to the nearest 0,01 g.

6.3 Procedure

6.3.1 Test portion

Weigh, to the nearest 0,01 g, approximately 3 g of the test sample.

NOTE 2 Since the mass of the test portion is low, it is advisable that it be taken from a homogenized sample.

6.3.2 Determination

Spread the test portion on a sheet of neutral grey paper. With the help of the small tongs (6.2.2), separate all the yellow filaments, attached or unattached, and other floral wastes that might be present.

Weigh on the analytical balance (6.2.3), to the nearest 0,01 g, the previously dried watch glass (6.2.1).

Transfer the separated floral wastes to the watch glass and weigh the whole to the nearest 0,01 g.

6.4 Expression of results

The floral waste content of the sample, expressed as a percentage by mass, is equal to

$$(m_2 - m_1) \times \frac{100}{m_0}$$

where

- m_0 is the mass, in grams, of the test portion;
- m_1 is the mass, in grams, of the watch glass;
- m_2 is the mass, in grams, of the watch glass containing the floral wastes.

7 Determination of extraneous matter content of saffron in filaments

7.1 Principle

Physical separation of the extraneous matter present in a test portion and weighing.

7.2 Apparatus

The same apparatus is required as in clause 6.

7.3 Procedure

7.3.1 Test portion

NOTE 3 Since the mass of the test portion is low, it is advisable that it be taken from a homogenized sample.

Reconstitute the test sample (approx. 3 g) by reincorporating the floral wastes previously separated and determined as in clause 6. Homogenize well and then weigh the sample to the nearest 0,01 g on the analytical balance.

7.3.2 Determination

Spread the test portion on a sheet of neutral grey paper. With the help of the small tongs, or with any other appropriate means, separate the extraneous matter from the test portion.

Weigh on the analytical balance, to the nearest 0,01 g, the previously dried watch glass.

Transfer the separated extraneous matter to the watch glass and weigh the whole to the nearest 0,01 g.

7.4 Expression of results

The extraneous matter content of the sample, expressed as a percentage by mass, is equal to

$$(m_3 - m_1) \times \frac{100}{m_0}$$

where

- m_0 is the mass, in grams, of the test portion;
- m_1 is the mass, in grams, of the watch glass;
- m_3 is the mass, in grams, of the watch glass containing the extraneous matter.

8 Microscopic examination of saffron in powder form

8.1 General

The method is applicable to the examination of saffron in powder form in order to determine whether the powder consists exclusively of vegetable elements belonging to *Crocus sativus* Linnaeus.

8.2 Principle

Verification of the identity and purity of the saffron powder and investigation for typical anatomical elements by microscopic observation, as described in 8.5. Examination of the sample in distilled water, in a sodium or potassium hydroxide solution and in aqueous iodine/iodide solution.

8.3 Reagents

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

8.3.1 Iodine/iodide solution, aqueous solution of iodine in potassium iodide.

To 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 water. Leave until completely dissolved, then make up to the mark with water. Stopper the flask.

8.3.2 Sodium or potassium hydroxide, aqueous solution, 5 % (m/m).

8.4 Apparatus

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

8.4.1 Microscope, capable of $\times 100$ to $\times 400$ magnification.

8.5 Procedure

8.5.1 Test portion

Take a test portion of the order of 0,001 g to 0,002 g, but the quantity may vary depending upon the sample to be analysed. If the typical elements are rare, it is recommended to mount (prepare) several slides.

8.5.2 Preparation for observation in water

This allows observation of all the elements of the powder.

Put a drop of water on a slide. With the tip of a scalpel or a lanceolate needle, take the test portion (8.5.1) and mix it in the water placed on the slide until the powder is thoroughly wet. Cover with a cover glass by pressing gently.

NOTE 4 The quantity of water to be deposited should enable the entire powder to be thoroughly wet, but it should not be in excess and run off the slide.

8.5.3 Preparation for observation in an aqueous solution of sodium or potassium hydroxide

This enables clarification of the preparations by destroying totally or partially the major part of the cellular contents, particularly starch. The cellular elements are also made clearer and easier to observe, particularly the sclerous elements, vessels, fibres and epidermis. The mineral elements are not altered.

Carry out the procedure indicated in 8.5.2 but replace the distilled water by the sodium or potassium hydroxide solution (8.3.2).

Wait for a few minutes for the medium to clarify.

8.5.4 Preparation for observation in aqueous iodine/iodide solution

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

Carry out the procedure indicated in 8.5.2 but replace the water with iodine/iodide solution (8.3.1).

8.5.5 Observation procedure

Place each of the slides prepared as in 8.5.2 to 8.5.4, in turn, under the microscope (8.4.1) with a magnification which can vary between $\times 100$ and $\times 400$, and proceed with the observation of the anatomical structure of the saffron (see 8.6).

NOTE 5 It is only by small details of structure, or even by comparison of their respective sizes, that it is possible to determine with certainty the source of a particular tissue.

8.6 Anatomical structure of saffron

8.6.1 Transverse section of a stigma

(see figure 1)

The section has the following parts:

- a parenchyma, formed of polygonal cells or cells with rounded corners, with thin walls;
- vascular bundles, of round cross-section;
- an epidermis composed of a row of slightly elongated plate cells perpendicular to the surface of the stigma and covered by a thin cuticle. Some epidermis cells have a small papilla in the middle of their outside wall.

8.6.2 Characteristics of saffron powder

The essential microscopic features characterizing saffron powder are as follows:

- fragments of the top extremity of the stigmas with large, hair-like elongated papillas capable of reaching a length of 150 μm (see figure 2);
- epidermic debris of stigmas with small round papillas (see figure 3);

- round pollen grains of large diameter (85 μm to 100 μm) with a thick, smooth cell wall and with a finely granular exine (see figure 4);

In addition, the following can also be observed (see figure 5):

- parenchymatous debris;
- debris of the epidermis of the style, consisting of long, thin-walled and slightly sinuous cells;
- debris of thin vascular bundles.

IMPORTANT — Saffron powder 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.

8.7 Interpretation of observations

After observation of the various slides, the examiner notes whether the saffron contains foreign elements or not.

If foreign elements are detected, they shall be compared with a standard reference preparation for identification purposes.

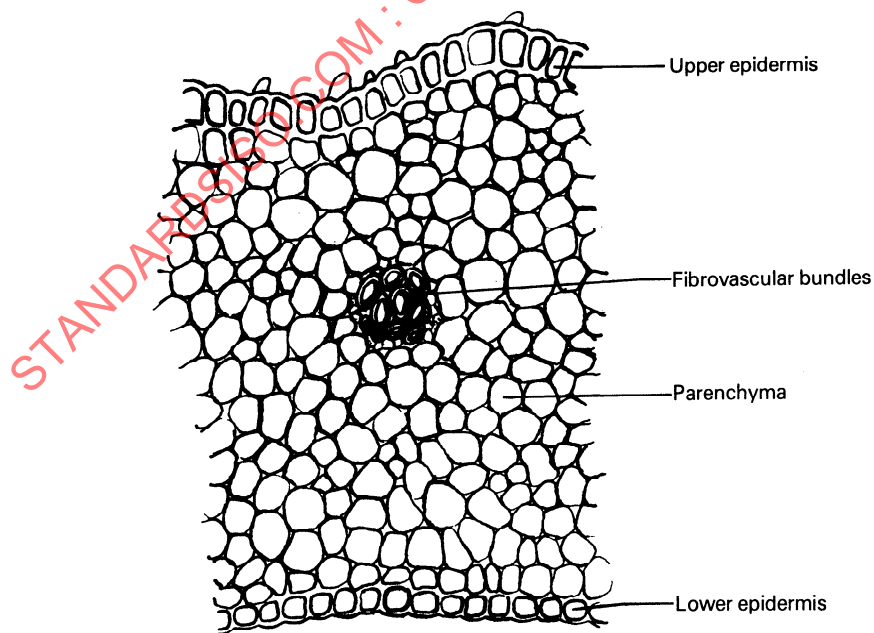


Figure 1 — Transverse section of stigma of saffron crocus

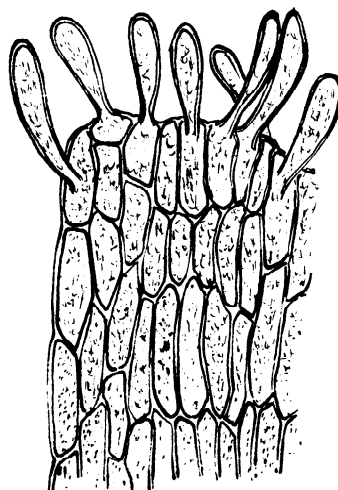


Figure 2 — Upper extremity of stigma of saffron crocus



Figure 3 — Upper epidermis of stigma of saffron crocus (front view)

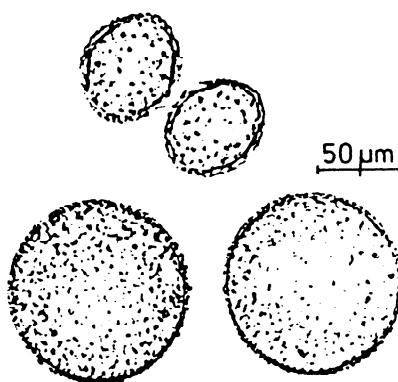


Figure 4 — Pollen grain of saffron crocus (magnification $\times 300$)

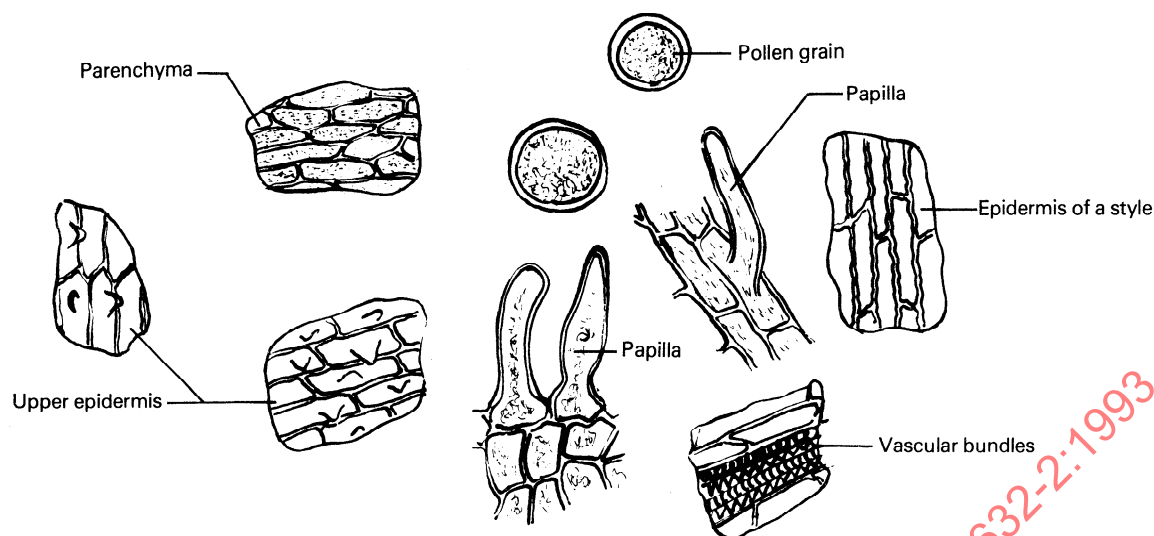


Figure 5 — Saffron in powder form

9 Determination of moisture and volatile matter content

9.1 General

This method is applicable to the determination of moisture and volatile matter content of saffron in filaments or in powder form.

NOTE 6 The method of determination of the moisture content of spices and condiments described in ISO 939¹⁾ is not applicable in the case of saffron because it requires the use of too large a test portion.

9.2 Principle

Oven drying at $103\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ to constant mass.

9.3 Apparatus

Usual laboratory apparatus and, in particular, the following.

9.3.1 Weighing dish or evaporating dish, provided with a lid or a watch glass.

9.3.2 Oven, capable of operating at $103\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$.

9.3.3 Desiccator, containing an effective desiccant.

9.3.4 Analytical balance, with an accuracy of 0,001 g.

9.4 Procedure

9.4.1 Test portion

9.4.1.1 Saffron in filaments

Carry out the operation with sample A (see table 1) as reconstituted after the determination and reincorporation of the floral wastes (clause 6) and extraneous matter (clause 7).

Weigh, to the nearest 0,001 g, into the weighing dish or evaporating dish (9.3.1), previously dried and tared to the nearest 0,001 g, approximately 2,5 g of sample A.

9.4.1.2 Saffron in powder form

Carry out the operation with sample A (see table 2).

Weigh, to the nearest 0,001 g, into the weighing dish or evaporating dish (9.3.1), previously dried and tared to the nearest 0,001 g, approximately 2,5 g of sample A.

9.4.2 Determination

Place the weighing dish or evaporating dish containing the test portion (9.4.1.1 or 9.4.1.2), uncovered, in the oven (9.3.2) set at $103\text{ }^{\circ}\text{C}$ and leave for 16 h. Cover with the lid or watch glass, and allow it to cool in the desiccator (9.3.3). After cooling, weigh to the nearest 0,001 g.

1) ISO 939:1980, *Spices and condiments — Determination of moisture content — Entrainment method*.

Keep the dry product for the later determination of total ash (see clause 10) and acid-insoluble ash (see clause 11).

Carry out two determinations on the same test sample.

9.5 Expression of results

The moisture and volatile matter content, expressed as a percentage by mass of the initial sample, is equal to

$$(m_0 - m_4) \times \frac{100}{m_0}$$

where

m_0 is the mass, in grams, of the test portion;

m_4 is the mass, in grams, of the dry residue.

Take as the result the arithmetic mean of the two determinations, if the conditions of repeatability are met.

10 Determination of total ash

This shall be in accordance with the method given in ISO 928.

For saffron in filaments or in powder form, use the sample which was used for the determination of the moisture content and take a test portion of 2 g.

11 Determination of acid-insoluble ash

This shall be in accordance with the method given in ISO 930.

For saffron in filaments or in powder form, use the sample which was used for the determination of total ash.

12 Crushing and sieving (for tests described in clauses 13 and 14)

12.1 Apparatus

12.1.1 Crusher, meeting the following requirements:

- easy to dismantle and to clean, and having a minimum dead space;
- permitting a quick and uniform crushing without causing heat or loss of moisture;

- avoiding contact with the ambient air as much as possible;
- permitting a total recovery of all the fragments of the sample;
- not introducing any extraneous substance into the sample.

12.1.2 Sieve, of 500 µm mesh.

12.2 Saffron in filaments

Crush sample B (see table 1) using the crusher (12.1.1) until 95 % of the product passes through the sieve (12.1.2).

12.3 Saffron in powder form

Sieve sample B (see table 2) using the sieve (12.1.2) in order to verify that 95 % of the powder passes through.

If this is not the case, crush the powder in the crusher (12.1.1) to obtain the required particle size.

13 Determination of the main characteristics (picrocrocin, safranal, crocine) — Spectrometric method

13.1 General

This method enables determination of the main characteristics of saffron connected with the picrocrocin, safranal and crocine content. It is directly applicable to saffron in powder form provided that the powder conforms to the requirements of 12.3, and to saffron in filaments after crushing and sieving in accordance with 12.2.

13.2 Principle

Recording the variation in optical density between 220 nm and 480 nm of an aqueous extract of saffron at ambient temperature.

13.3 Apparatus

Usual laboratory equipment and, in particular, the following.

13.3.1 Spectrometer, suitable for recording optical density in the ultraviolet band between 220 nm and 480 nm.

13.3.2 Silica cell, with an optical path length of 1 cm.

13.3.3 Volumetric flasks, class A, in antiactinic glass, of capacity 200 ml and 1 000 ml.

13.3.4 Pipette, class A, of capacity 20 ml.

13.3.5 Filtration membrane, made of cellulose acetate, of 50 mm diameter and porosity 0,45 µm.

13.4 Procedure

13.4.1 Test portion

Weigh exactly 500 mg of sample B (table 1 or 2) to the nearest 0,001 g in a watch glass.

NOTE 7 The extraction should be carried out on a sample that has not been subjected to any prior treatment.

13.4.2 Determination

Transfer quantitatively the test portion into the 1 000 ml volumetric flask (13.3.3). Add about 900 ml of distilled water (analytical grade).

Stir with a magnetic stirrer (1 000 r/min) for 1 h, away from light. Remove the magnetic bar.

Make up to the mark with distilled water. Close with a glass stopper and homogenize.

Take an aliquot part with the 20 ml pipette (13.3.4). Transfer into the 200 ml volumetric flask (13.3.3). Adjust to the mark with distilled water. Close with a glass stopper and homogenize.

Filter the solution, rapidly and away from light, through the membrane (13.3.5) so as to obtain a clear solution.

Adjust the spectrometer (13.3.1) and record the variation in absorbance of the filtered solution between 220 nm and 480 nm using distilled water as the reference liquid.

13.5 Expression of results

The results are obtained by direct reading of the absorbance at three wavelengths, corresponding to the maximum absorptions of picrocrocine, safranin and crocine as follows:

picrocrocine: absorbance $E_{1\text{ cm}}^{1\%}$ at about 257 nm

safranin: absorbance $E_{1\text{ cm}}^{1\%}$ at about 330 nm

crocine: absorbance $E_{1\text{ cm}}^{1\%}$ at about 440 nm

13.6 Test report

The test report shall specify the method used and the results obtained. It shall also mention all operating details not specified or regarded as optional, as well as any incidents which may have influenced the results.

It should in particular specify

- the moisture content as determined by the method described in clause 9;
- the grain size of the saffron if it is in powder form.

The test report shall include all information necessary for the complete identification of the sample.

14 Identification of saffron pigments

14.1 General

This method is carried out directly on saffron in powder form provided that the powder conforms to the requirements of 12.3, and on saffron in filaments after crushing and sieving in accordance with 12.2. It reveals the pigments existing in the saffron, and these specific constituents can be used as an indication of the authenticity of the product.

14.2 Principle

After soaking the saffron in methanol, thin-layer chromatography of the methanol solution. Examination of the plate after development and identification of the pigments to determine whether they are specific to saffron or not.

14.3 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled water or water of equivalent purity.

14.3.1 Methanol, boiling point 64 °C to 65 °C at 103,3 kPa (760 mmHg), density 20/20 = 0,791 to 0,793.

14.3.2 Ethanol, absolute, 99,5 % (V/V) min.

14.3.3 Chloroform, boiling point about 60 °C at 103,3 kPa (760 mmHg), density 20/20 = 1,475 to 1,081. This quality of chloroform shall not contain more than 2 % (V/V) of ethanol.

NOTE 8 Dichloromethane may also be used.

14.3.4 Sulfuric acid, concentrated, 95,0 % (m/m) minimum and 97,0 % (m/m) maximum.

14.3.5 4-Methoxybenzaldehyde.

14.3.6 Naphthol yellow, monosodium salt of 2,4-dinitronaphthol²⁾.

14.3.7 Sudan red G, 2-hydroxy-[1-(2-methoxy)phenylazo]naphthalene³⁾.

14.3.8 Reference solution, prepared by dissolving 5 mg of Naphthol yellow (14.3.6) in 5 ml of methanol (14.3.1), and adding a solution of 5 mg of Sudan red G (14.3.7) in 5 ml of chloroform (14.3.3).

14.3.9 Elution solvent, consisting of the organic phase of a mixture containing.

— ethyl acetate (65 volumes), boiling point 76 °C to 78 °C at 103,3 kPa (760 mmHg);

— propan-2-ol (25 volumes), and

— water (10 volumes).

14.3.10 Revealing solution, prepared by mixing in the following order:

— 10 ml of 4-methoxybenzaldehyde (14.3.5),

— 90 ml of ethanol (14.3.2), and

— 10 ml of sulfuric acid (14.3.4).

14.4 Apparatus

Usual laboratory equipment and, in particular, the following.

14.4.1 Test tube, of dimensions 60 mm × 7 mm.

14.4.2 Microsyringe or micropipette, permitting delivery of 5 µl and 10 µl.

14.4.3 Chromatography cell.

14.4.4 Silica gel plates, with indicator of fluorescence GF 254.

14.4.5 Glass wool plug.

14.5 Procedure

14.5.1 Test portion

Weigh about 0,05 g of sample B (see table 1 or 2) to the nearest 0,01 g.

14.5.2 Preparation of solution

Introduce the test portion (14.5.1) into the test tube (14.4.1) and moisten it with a drop of water. Wait for 2 min to 3 min, then add 1 ml of methanol (14.3.1). Allow the solution to settle for 29 min away from light. Filter it on a small glass wool plug (14.4.5).

14.5.3 Operating technique

Using the microsyringe or the micropipette (14.4.2), deposit separately in bands of length 2 cm to 4 cm on a silica gel plate (14.4.4) 5 µl of the solution to be examined (14.5.1) and 5 µl of the reference standard solution (14.3.8). Develop in the cell (14.4.3) with the elution solvent (14.3.9) until the solvent front has progressed 10 cm. Allow the solvent to evaporate.

Examine the chromatogram in ultraviolet light at 254 nm, then in daylight.

Then spray onto the plate about 10 ml of the revealing solution (14.3.10).

Heat for 5 min to 10 min at a temperature of 105 °C to 110 °C while observing the chromatogram.

14.6 Interpretation of results

14.6.1 Observation in daylight

The lower third of the chromatogram shows three yellow spots. The spot on the bottom strip is of more intense colour, and corresponds in colour and in size to the Naphthol yellow spot. It characterizes crocine.

14.6.2 Observation in ultraviolet light

The chromatogram observed in ultraviolet light at a wavelength of 254 nm shows four main fluorescent spots, three corresponding to the spots observed in daylight and another with a higher R_f value (around 0,55), which characterizes picrocrocine.

One or two rather faint spots of fluorescence are visible at the level of the Sudan red G, characterizing β -hydroxycyclocitral and safranal.

2) Colour index No. 10315; Schultz No. 18.

3) Colour index No. 12150; Schultz No. 149.

After spraying with the revealing solution (14.3.10)

- the crocine becomes greyish green in colour, and
- the picrocrocine becomes violet in colour.

The chromatogram shall not show any other colour spots (particularly yellow-orange or red spots) before spraying, particularly at the starting point. These would correspond to a deterioration of the crocine and/or the presence of foreign colouring matter.

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