International Standard 3632

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Saffron — Specification

Safran — Spécifications

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Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 3632 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in December 1978.

It has been approved by the member bodies of the following countries :

Brazil	Hungary	South Africa, Rep. of
Bulgaria	India	Spain
Chile	Israel	Thailand
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The member body of the following country expressed disapproval of the document on technical grounds :

Netherlands

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Saffron — Specification

1 Scope

This International Standard specifies requirements for saffron obtained from the flowers of *Crocus sativus* Linnaeus.

2 Field of application

This International Standard is applicable to saffron in either of the following forms :

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

— in powder form, obtained by grinding saffron filaments.

It is not applicable to saffron occurring in cut filaments, i.e. in the form of stigmas deprived of the style.

3 References

ISO 927, *Spices and condiments — Determination of extraneous matter.*

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 1871, *Agricultural food products — General directions for the determination of nitrogen by the Kjeldahl method.*

ISO 5498, *Agricultural food products — Determination of crude fibre content — General method.*[1)]

4 Definitions

4.1 saffron in filaments : Stigmas of *Crocus sativus* Linnaeus, dried, dark red in colour and rolled into cornets, serrated or indented at the distal end. The stigmas may be either isolated or joined in twos or threes at the end of a portion of the style (which is also red in colour).

4.2 yellow filaments : Dried yellow stigmas of flowers of *Crocus sativus* Linnaeus.

4.3 floral waste : Yellow filaments, pollen, stamens, parts of ovary and other parts of the flower of *Crocus sativus* Linnaeus.

4.4 extraneous matter : Leaves, stems, chaff and other vegetable matter. The only mineral matter permitted is sand, earth and dust.

1) At present at the stage of draft.

5 Specification

5.1 Classification of saffron in whole filaments

Saffron in whole filaments is classified into three categories, as shown in table 1, on the basis of its floral waste and extraneous matter contents, which are determined respectively according to the method specified in annex B and that specified in ISO 927.

Table 1 — Classification of saffron in whole filaments

Characteristic	Category I type "Mancha"	Category II type "Rio"	Category III type "Sierra"
Floral waste, % (m/m) max.	7	13 to 15	17 to 20
Extraneous matter, % (m/m) max.	0,5	1	1

5.2 Flavour

The flavour of saffron shall be specific, slightly bitter and a little pungent. The product shall be free from foreign flavours.

5.3 Freedom from moulds, insects, etc.

Saffron shall be free from living insects, and shall be practically free from moulds, dead insects, insect fragments and rodent contamination visible to the naked eye (corrected, if necessary, for abnormal vision) using the required magnifying instrument in each particular case. If the magnification exceeds X 10, this fact shall be mentioned in the test report.

5.4 Chemical requirements[1)]

Saffron in filaments or in powder form shall comply with the requirements laid down in table 2.

Saffron in powder form, when examined by the method specified in annex E, shall not reveal the presence of pigments other than those which are peculiar to saffron.

Table 2 — Chemical requirements for saffron in filaments or in powder form

Characteristic	Requirement		Method of test
	Saffron in filaments	Saffron in powder form	
Water and volatile matter, at 103 °C, % (m/m) max.	14	8	Annex C
Total ash, % (m/m), on the dry basis :			ISO 928
max.	8	8	
min.	5	5	
Ash insoluble in HCl, % (m/m), on the dry basis :			ISO 930
Category I, max.	1,0	1,0	
Categories II and III, max.	1,5	1,5	
Extract soluble in cold water, % (m/m), on the dry basis :			ISO 941
max.	65	65	
min.	55	55	
Total nitrogen, % (m/m), on the dry basis :			ISO 1871
max.	3,0	3,0	
min.	2,0	2,0	
Difference between the percentages on the dry basis, of reducing sugars before and after inversion, expressed in invert sugar, max.	*	*	
Crude fibre, % (m/m), on the dry basis, max.	**	**	ISO 5498
Colouring power $E^{1\,\%}_{1\,cm}$ at 440 nm :			Annex D
Category I, min.	110***	150***	
Category II, min.	80***	120***	

* Requirements will be indicated when a method has been standardized.

** Limits will be fixed later according to the method used.

*** Value given on an experimental basis.

1) Limits for toxic substances will be included later, in accordance with the recommendations of the FAO/WHO Codex Alimentarius Commission.

6 Sampling

6.1 Sample the product by the method specified in ISO 948.

6.2 Minimum mass of laboratory sample : 20 g in the case of saffron in filaments; 10 g in the case of saffron in powder form.

NOTE — In order to carry out all the test methods described in this International Standard, a sample of at least 25 g is required.

7 Methods of test

An analysis shall be carried out on the saffron samples to ensure that they are in conformity with the specifications of this International Standard; the analysis shall be carried out according to the methods of test indicated in clause 5.

For the determination of extraneous matter, proceed as specified in ISO 927, but use sample A (at least 10 g; see annex A, clause A.1).

A microscopic examination is also recommended in the case of saffron in powder form (see annex F).

8 Packing and marking

8.1 Packing

Saffron in filaments and in powder form shall be packed in rigid, water-tight, sound and clean containers which shall be of a material that can have no influence on the saffron.

8.2 Marking

8.2.1 Saffron in filaments

The following special indications shall be marked on each package to be dispatched, or on a label :

a) name of the product (botanical name), the mention "whole filaments", and the commercial name or brand, if any;

b) name and address of the producer or packer;

c) batch or code number;

d) net mass;

e) category of the product;

f) name of the producing country;

g) any other indication required by the buyer, such as year of harvest and date of packing (if known).

8.2.2 Saffron in powder form

The indications a) to d) given in 8.2.1, shall be marked on each unitary container. If glass containers are used, the words "Fragile - Glass" shall be marked on each package to be dispatched.

Annex A

Preparation of test sample

A.1 Saffron in whole filaments

Divide the laboratory sample, which shall have a mass of at least 20 g, into two equal samples A and B, each with a mass of at least 10 g, and intended for :

a) Sample A :

— identifying the product,

— evaluating the flavour,

— verifying freedom from moulds, insects, etc.,

— determining the proportion of floral waste,

— determining the proportion of extraneous matter.

b) Sample B.

— various other tests including, in the first instance, the determination of water and volatile matter content in the product as received, and then preparation of the powdered test sample.

A.2 Saffron in powder form

Saffron in powder form is analysed without any prior preparation.

Annex B

Determination of percentage of floral waste

Weigh 5 g of saffron from sample A (see annex A). Spread out the product on a sheet of white paper and by means of small tweezers pick out all the attached or free yellow filaments, and other floral waste which may be present. Place the remainder on a tared watch glass and weigh on an analytical balance. The difference between the two masses gives the quantity of floral waste in the quantity of product weighed.

Calculate from this the percentage by mass.

Annex C

Determination of moisture and volatile matter content

C.1 Principle

Oven drying at 103 ± 2 °C to constant mass.

C.2 Apparatus

Usual laboratory equipment, not otherwise specified, and the following items :

C.2.1 Weighing dish, fitted with a lid or watch glass.

C.2.2 Oven, capable of being regulated at 103 ± 2 °C.

C.2.3 Desiccator, containing an effective desiccant.

C.2.4 Analytical balance.

C.3 Procedure

C.3.1 Test portion

Weigh, to the nearest 1 mg, into the weighing dish (C.2.1), previously dried and tared to the nearest 1 mg, either approximately 5 g of sample B of saffron in whole filaments (see annex A) or approximately 2,5 g of saffron powder, as the case may be.

C.3.2 Determination

Place the weighing dish containing the test portion, uncovered, in the oven (C.2.2) regulated at 103 ± 2 °C and leave for 16 h. Cover with the lid or watch glass, and allow it to cool in the desiccator (C.2.3). After cooling, weigh to the nearest 1 mg.

Keep the dry product for later determinations.

Carry out two determinations on the same test sample.

C.4 Expression of results

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

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

where

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

m_1 is the mass, in grams, of the dry residue.

Take as the result the arithmetic mean of the two determinations.

Annex D

Determination of colouring power

D.1 Definition

colouring power of saffron : The specific extinction of an aqueous extract of the product at 440 nm.

D.2 Principle

Dilution of the cold water soluble extract obtained according to ISO 941, so as to bring the concentration to about 4 mg of saffron per 100 ml of solution. Measurement of the absorbance at 440 nm in a cell of 1 cm optical path length.

D.3 Apparatus

Usual laboratory equipment, not otherwise specified, and the following items :

D.3.1 Pipette, of capacity 1 ml, conforming to class A of ISO 648 or ISO/R 835.

D.3.2 Volumetric flask, of capacity 500 ml, conforming to class A of ISO 1042.

D.3.3 Spectrophotometer (or any other apparatus capable of measuring absorbance at 440 nm).

D.3.4 Cells for spectrophotometry, of vitreous silica, transparent to ultra-violet light, having an optical path length of 1 cm.

D.4 Procedure

D.4.1 Test portion

Use the clear supernatant liquid obtained before filtration during the determination of the cold water soluble extract.[1)]

From this solution, which contains 2 g of saffron per 100 ml, take with the pipette (D.3.1) a volume of 1 ml.

D.4.2 Determination

Transfer the test portion into the 500 ml volumetric flask (D.3.2) and dilute to the mark with distilled water so as to obtain a solution containing about 4 mg of saffron per 100 ml of solution.

Adjust the spectrophotometer (D.3.3). Measure the absorbance of the final dilution at 440 nm in a cell (D.3.4) using distilled water as the reference liquid.

D.5 Expression of results

Note the absorbance measured at 440 nm and calculate the specific extinction $E^{1\,\%}_{1\,cm}$ at 440 nm as a function of the dilution obtained.

1) It is essential to take the clear supernatant liquid before filtration because filter paper strongly adsorbs the saffron pigments.

Annex E

Investigations of saffron pigments

E.0 Introduction

The pigments present in saffron are specific constituents and can be used as an indication of the authenticity of the product.

E.1 Principle

After maceration of the saffron with ethanol, thin layer chromatography of the ethanolic solution.[1)]

E.2 Reagents

E.2.1 Ethanol, 80 % (*V*/*V*) solution.

E.2.2 Elution solvent, constituted by the organic phase of a mixture containing :

— butan-1-ol (4 volumes);

— acetic acid (1 volume);

— water (1 volume).

This reagent has a limited stability.

E.3 Apparatus

Usual laboratory equipment, not otherwise specified, and the following items :

E.3.1 Cell for chromatography, having its inner walls covered by filter paper in order to ensure a saturated atmosphere.

E.3.2 Silica plates, without fluorescence indicator, activated by heating for 1 h at 103 ± 2 °C and cooling in a desiccator containing an effective desiccant.

E.4 Procedure

E.4.1 Test portion

Weigh, to the nearest 0,01 g, about 0,05 g of the laboratory sample.

E.4.2 Determination

Add to the test portion 2 ml of the ethanol (E.2.1) and allow it to undergo maceration for about 2 h. Deposit 1 µl of the ethanolic solution thus obtained on a silica plate (E.3.2). Develop in the cell (E.3.1) in the elution solvent (E.2.2) until the elution solvent front has progressed at least 10 cm from the starting line (but never beyond the total length of the plate).

E.5 Interpretation of results

After development, the pigments peculiar to saffron show themselves in the manner indicated in table 3.

Table 3 — Evidence of pigments peculiar to saffron

R_f (relative to the front) (approximate value)	Intensity of the spots	Colour of the spots	
		in daylight	in ultra-violet light
0,96	very faint spot	Yellow-orange	
0,80	very faint spot	Yellow-orange	
0,63		Yellow-orange	
0,56	clear spot	Yellow-orange	Brown
0,43	clear spot	Yellow-orange	Brown
0,29	significant spot	Yellow-orange	Brown

The three spots of R_f 0,29, 0,43 and 0,56 are the principal spots and characterize the stigmas of pure saffron. They are yellow-orange coloured in daylight (orange if significant quantities are present). All three give a brown fluorescence in ultra-violet light. The substance with the lowest R_f value (0,29) is *trans*-crocine.

Note the presence of other spots, if any, which do not correspond to saffron pigments.

1) Any other equivalent method can also be used.

Annex F

Microscopic determination of saffron in powder form

F.1 General method for preparing vegetable species in the powder state for microscopic observation

F.1.1 Principle

Separation of the cells from their protoplasmic content in order to permit easier observation of the distinguishing characteristics. To achieve this, it is generally necessary to subject the sample to alkali treatment by a 100 g/l sodium hydroxide solution.

F.1.2 Procedure

Suspend a test portion of the ground sample, if necessary extracted by diethyl ether, in a sodium hydroxide solution and then subject to gentle boiling for 10 to 15 min.

NOTE — The concentration of the sodium hydroxide solution and the boiling period depend on the extent of the alkali treatment, and are left to the discretion of the operator.

Add a sufficient quantity of water to the treatment solution to reduce the density of the liquid and to permit the deposition of all suspended cellulose material. Pour off the alkaline solution and wash the residue obtained two or three times by decantation with tepid water. Carry out these operations in stemmed glasses.

In the case of elements which are difficult to collect, use centrifugation.

Take fragments of tegument directly using a lance-shaped instrument, and place between a slide and cover slip[1] in glycerized water. Observation can be improved by mounting the preparation in a clarifying solution such as Amann lactophenol[2].

F.2 Anatomical structure of saffron

F.2.1 Transverse section of a stigma (see figure 1)

This shows :

— a parenchyma, formed from cells which are polygonal or rounded on their angles, with walls of low thickness;

— vascular bundles, with rounded section;

— an epidermis, composed of a series of cells arranged in rows, slightly elongated perpendicularly to the surface of the stigma and covered with a cuticle of low thickness. Some of the cells of the epidermis have a small papilla in the centre of their external wall.

F.2.2 Characteristics of the powder

Observations are usually made at magnifications of 100 to 400 diameters. The origin of a particular tissue can only be determined for certain by means of small structural details or even by comparison of their relative dimensions.

If foreign elements are detected, a reference preparation must be examined.

The main microscopic characteristics which distinguish saffron are as follows :

— fragments of the distal part of the stigmata with large papillae which are elongated like hairs (see figure 2);

— debris of the epidermis of the stigmata with small papillae (see figure 3);

— smooth grains of pollen (see figure 4).

1) During the preparation of the slide, it is recommended that the slide and cover slip be warmed to assist in the expulsion of entrapped air bubbles.

2) The Amann lactophenol solution may be prepared as follows.

CAUTION — It is essential that the preparation of the solution is carried out under a ventilated hood and that suitable precautions are taken during its use.

Melt 20 g of phenol crystals in a suitable container, on a water bath, then add slowly, and with constant stirring, 20 g of lactic acid (ϱ_{20} = 1,20 g/ml), 40 g of glycerine (ϱ_{20} = 1,50 g/ml) and 20 ml of distilled water.