International Standard 7542

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Ground (powdered) paprika (*Capsicum annuum* Linnaeus) — Microscopical examination

Paprika (Capsicum annuum *Linnaeus*) en poudre — *Examen au microscope*

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Foreword

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Ground (powdered) paprika (*Capsicum annuum* Linnaeus) — Microscopical examination

1 Scope and field of application

This International Standard describes the morphological and anatomical structure of paprika (*Capsicum annuum* Linnaeus) and specifies a method for the microscopical examination of ground (powdered) paprika.

2 Reference

ISO 7540, *Ground (powdered) paprika* (Capsicum annuum *Linnaeus) — Specification.*

3 Definitions

3.1 paprika : The whole fruit of paprika, consisting of the pericarp, the seeds, the peduncle, the calyx and the placenta.

3.2 ground [powdered] paprika : The product obtained by grinding varieties of dried ripe paprika.

4 Structure of paprika

4.1 Description and morphological structure

Paprika is an inflated berry. Several varieties of paprika having different shapes, are cultivated. Ground (powdered) paprika is produced from long or short cylindro-conical varieties.

The pericarp is relatively thin, slightly juicy, coriaceous on the outside, and with a smooth red or dark red surface when mature.

The fruit is loculated by a septum into two, three or, sometimes, four parts which are well developed in the lower part of the fruit and flatten towards the apex, so that the upper part of the fruit is unilocular.

The semi-spherical shaped central placenta is in the lower part of the fruit where the majority of the seeds develop. Seeds appear to a lesser extent on the septum. The seeds are 2,8 to 4,5 mm in length, flat ovoid or kidney shaped and thicker at the edges. The open-edged hilum is at the ventral side of the seed. The seeds are of a yellowish-white or yellowish-brown colour, with a fine, glittering surface, slightly darker towards the edges.

The calyx is attached to the lower part of the fruit; its shape is characteristic of the variety. In general, it is formed by five fused peaked sepals. The calyx is a prolongation of the peduncle, of which the shape and the length are characteristic of the variety. The fruit may be upright, hanging or side-ways.

The placenta, the calyx and the peduncle are together called the "stalk".

4.2 Anatomical structure (see figure 1)

The anatomical structure of paprika can be more or less recognized in the ground product. The cells of the epidermis (exocarp) of the pericarp are flat and polygonal when viewed from above, the walls being thicker at the corners and slightly pitted. Their outer surface is covered with a thick cuticula, where sharp, transverse furrows can be observed, especially in vertical sections.

In the mesocarp, two parts can be distinguished : on the outside, the hypodermis adjoining the epidermis, and on the inside, the fleshy pericarp.

The hypodermis is uni- or multi-seriate : its cells, like the epidermis, are thicker at the corners and are slightly pitted, the walls being suberized and yellow. Inside the hypodermis, there is a layer of wider parenchyma cells whose walls become progressively thinner, but which are not suberized and in which, in varieties of paprika which are red when mature, contain red chromatoplasts. When not quite mature, globoid starch particles may be seen.

Dispersed throughout the fleshy pericarp, fibro-vascular bundles of ringed, spiral and spotted vessels can be seen.

On the inner side of the mesocarp, so called "giant" cells, of diameter 1 to 2 mm, can be seen, separated by layers of thin walled cells which cannot be recognized in the ground product.

The endocarp, composed of a single layer of cells, is immediately adjacent to the giant cells. This consists of two kinds of cells :

a) thick walled, yellow "bead string" sclerous cells, which are more or less elongated and parallel to the axis of the fruit, and which appear undulated when viewed from above; they are slightly lignified and grouped under the giant cells;

b) elongated, unlignified, thin walled cells separating the grouped cells mentioned in a), forming a continuous network which can be easily torn. Only their fragments can be detected in the ground product.

The cells of the septum are similar to those of other parts of the fleshy pericarp. They do not contain chromatoplasts, the vascular bundles are thicker and there are more spiral elements with wider cavities. The pungent principle of paprika, capsaicin, develops in the glandular cells located in groups in the epidermal tissue of the septum. Glandular cells containing capsaicin are not identifiable in the ground product. Neither the seeds nor the pericarp contain capsaicin.

The central placenta consists mainly of colourless thin walled parenchyma cells similar to those of the fleshy pericarp and the septum. The vascular bundles are strongly developed. In some places, very characteristic stone cells with slightly lignified walls can be seen among the thin walled parenchyma cells.

The surface of the seeds is covered by a hard yellowish seed coat, the outward layer of which is the epidermis, consisting of large greenish-yellow cells. The radial walls of the cells, as viewed from above, are strongly undulated (mesentery cells) and, together with the inner walls, are considerably thickened and highly lignified with slit-shaped pits.

The thickness and height of the cell walls increase towards the edges of the seed and decrease towards the flat surface of the seed. The outer cell wall is thin, more or less convex, unlignified, and it is only in the innermost part that a very thin layer of cells with lignified walls can be found. Under the epidermis (exocarp) are the parenchyma cells, next to which is the thicker endocarp which consists of cells with lignified walls. The seed protein tissue consists of comparatively thick walled polygonal cells of uniform diameter (isodiametric cells) containing oil and aleurone.

The upper epidermis (on the side closest to the fruit) of the five pointed-sepaled calyx consists of cells with slightly undulated walls. The upper epidermis carries short glandular hairs consisting of a dark red plurilocular ovoid gland supported on a short peduncle.

The cells of the lower epidermis are larger, octagonal and are interpersed with stomata but have no glandular hairs. Between the two epidermes, there is a spongy parenchyma, with spiral vascular bundles, green chromatoplasts and calcium oxalate crystals.

The peduncle of the paprika is covered by an epidermis containing a green colouring material which turns to brown at maturity. Under the epidermis, vascular tissue can be seen in the parenchyma cells with thin walls. The elements of the xylem (of the vascular bundles) are lignified, with bordered pits showing reticular thickening. The elongated thick walled xylem fibres with slit-like pits and the fibres of the phloem serve as supporting tissues.

Parenchyma cells are found next to the long fibres in the phloem.

5 Microscopical examination

5.1 Principle

Clarification of a pinch of ground paprika on a microscope slide and examination of particles under appropriate magnification.

5.2 Reagents

All reagents shall be of recognized analytical grade and the water used shall be distilled water or water of at least equivalent purity.

5.2.1 Glycerol, solution.

Dilute one part by volume of glycerol with one part by volume of water.

5.2.2 Sodium hypochlorite, solution.

Mix five volumes of sodium hypochlorite solution with two volumes of hot water.

5.2.3 Iodine-potassium iodide solution (Lugol solution).

Dissolve 2 g of potassium iodide and 1 g of iodine in a little water, and dilute to 100 ml.

5.2.4 Iodine-sodium hypochlorite solution.

Add iodine to the sodium hypochlorite solution (5.2.2) until a brown colour is obtained.

5.3 Apparatus

5.3.1 Binocular microscope, with magnification of X 50 to X 1 300, fitted with a filar micrometer eyepiece and a built-in lamp.

5.3.2 Microscope slides.

5.3.3 Cover slides.

5.3.4 Watch-glass.

5.3.5 Mounted needle.

5.3.6 Scalpel.

5.4 Preparation of the sample

Prepare several samples for microscopical examination. Place a pinch of ground (powdered) paprika on a microscope slide (5.3.2), mix carefully with the clarifying solutions and cover with a cover slide (5.3.3).

Glycerol solution (5.2.1) makes the preparation light and more translucent; it slightly dissolves colouring matter, and colourless parts become translucent.

Sodium hypochlorite has a bleaching effect making coloured particles become pale and it dissolves a great deal of the contents of the cell, rendering the preparation more translucent.

This bleaching effect can be accelerated by carefully heating the slide until the sodium hypochlorite solution begins to boil, but replacing the water lost through evaporation.

The iodine-potassium iodide solution is used most usually for the detection of foreign starch. The pericarp of ripe paprika does not contain starch.

The iodine-sodium hypochlorite solution is used for the detection of fine starch particles from unripe pericarp. The reagent renders the material clear, swelling starch particles and colouring them blue.

5.5 Procedure

The examination is usually carried out under a magnification of X 200 to X 600. In some cases, the origin of a particular tissue can only be determined by seeking small structural details, or by comparison of relative dimensions. (See figure 1 for the transverse section of the pericarp of paprika.)

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

The main microscopical characteristics which distinguish paprika are as follows :

a) exocarp (epidermis) of the pericarp (see figure 2);

b) "bead string" sclerous cells of the paprika endocarp (see figure 3);

c) epidermis of the paprika seed (mesentery cells) (see figure 4);

d) epidermis of the calyx (see figure 5);

e) stone cells of the placenta (see figure 6);

f) elements of the peduncle (see figure 7);

g) large numbers of red oil drops.

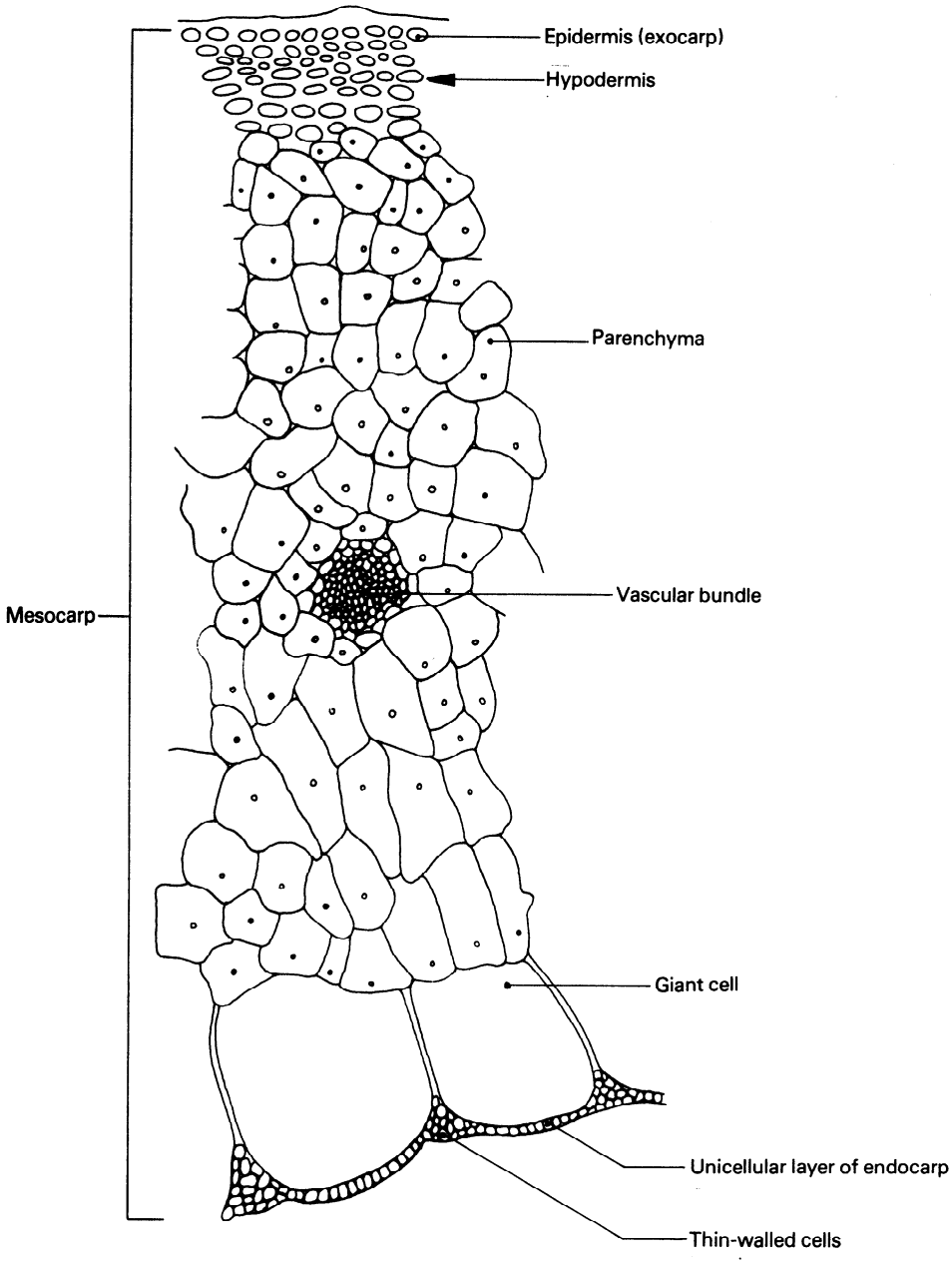

Figure 1 — Transverse section of paprika pericarp

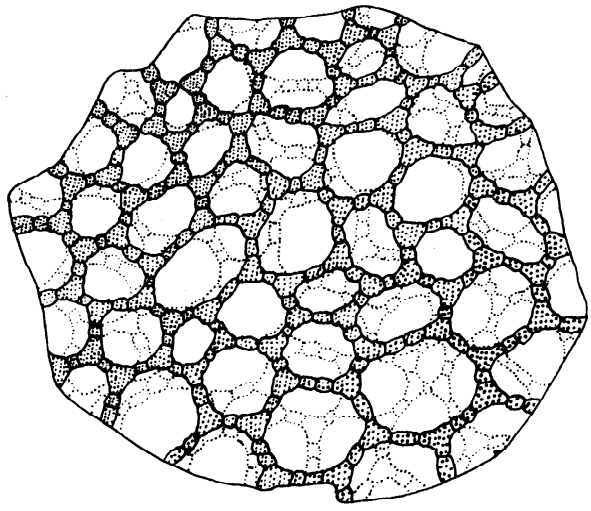

Figure 2 — Exocarp of the pericarp of paprika

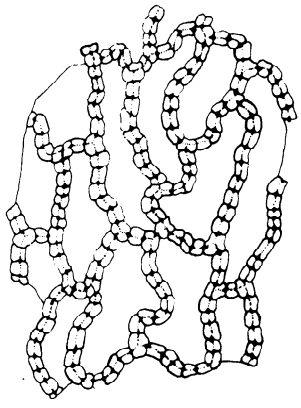

Figure 3 — "Bead string" cells of the endocarp of the pericarp of paprika, seen from above

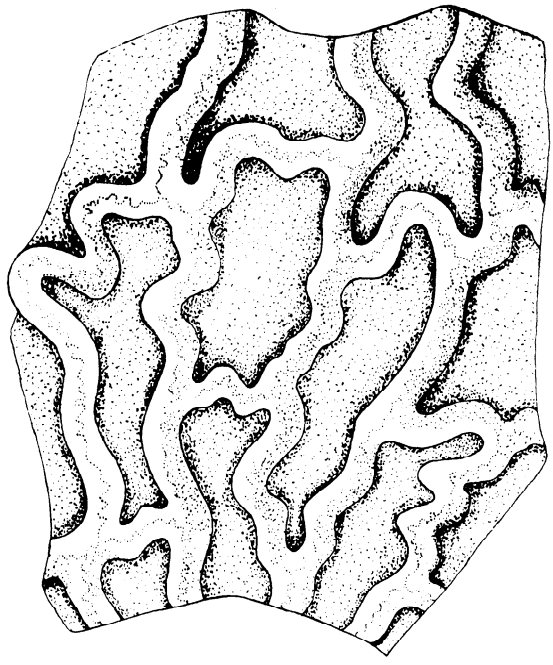

Figure 4 — Pericarp of the paprika seed (mesentery cells), seen from above

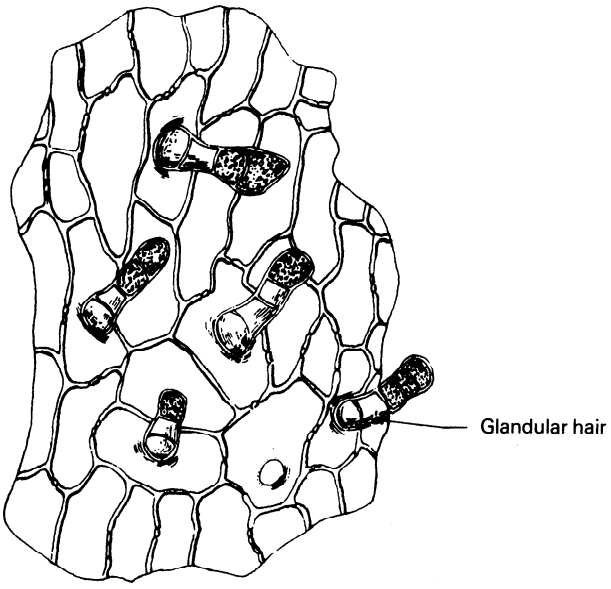

Figure 5 — Epidermis of the calyx

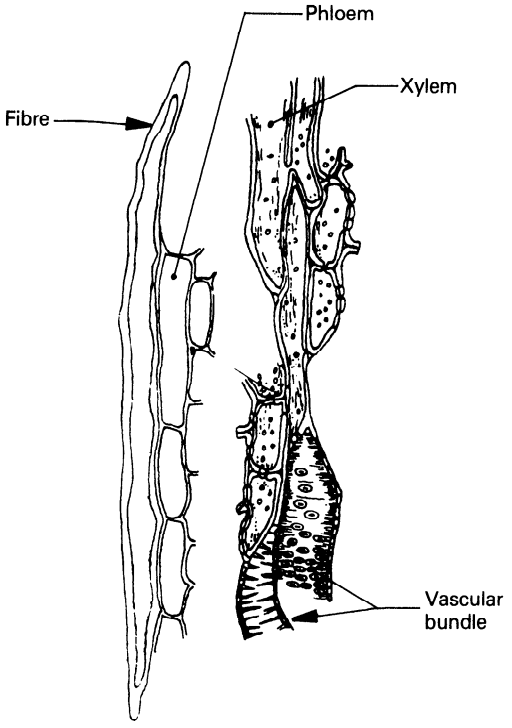

Figure 7 — Elements of the peduncle

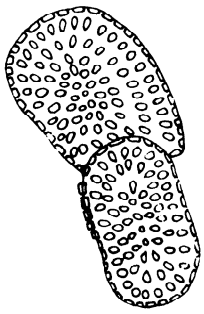

Figure 6 — Stone cells of the placenta