REVIEW ARTICLE

SCLERENCHYMA IN THE DIAGNOSIS AND ANALYSIS OF VEGETABLE POWDERS

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Introduction.

Sclerenchyma is the name given to any hard vegetable tissue other than vascular tissue. There are two types of sclerenchyma, *viz.*:—(a) parenchymatous sclerenchyma, the cells of which are termed sclereids or stone-cells, and (b) prosenchymatous sclerenchyma, the cells of which are termed fibres. Both sclereids and fibres have heavily thickened walls which are usually lignified; in a few plants, however, cellulosic sclerenchyma occurs, as in the endosperm of the date, *Phœnix dactylifera* Linn., and in that of the corozo or vegetable ivory nut, *Phytelephas macrocarpa* Ruiz et Pav., which are composed of sclereids, and in the pericycle of flax and hemp and the phloem of mezereon and slippery elm barks, in which the fibres are unlignified. The striking appearance and, when lignified, the strong staining reactions of these cells render them easily identifiable and, since as long as a century ago, they have been regularly used as a means of identifying vegetable materials, such as tea (sclereids) and cinchona barks (fibres), (see Fig. 1 R and G).

The identifications were based at first upon the form, abundance and manner of distribution of the sclerenchyma. Measurements were not usually given for sclereids and only rarely for fibres, although they were used for starches and blood-corpuscles and for materials like lupulin and lycopodium which are composed of discrete particles. Towards the end of the nineteenth century measurements of the length and width of cells in sclerenchyma began to be made as a routine addition to the verbal descriptions. The dimensions given were used chiefly as a record of observed facts and also for the purpose of making drawings to scale. Greenish (1903) in his "Foods and Drugs" and Greenish and Collin (1901 to 1904) in their "Anatomical Atlas" advocate and use measurements for starches, but only rarely for crystals of calcium oxalate, e.g., in rhubarb, squill, and orris, and very occasionally for the dimensions of cells; otherwise only vague statements such as "very large cells", "short hairs", "small rosettes", etc., are made about dimensions. Tschirch and Oesterle (1890) introduced many measurements into their "Anatomischer Atlas" and, about the same date, measurements began to be used for the characterisation of certain drugs, such as the different cinchonas and for the exclusion of particular adulterants, such as cassia bark when substituted for cinnamon. Ludwig Koch in his atlas "Die mikroskopische Analyse der Drogenpulver" 1900-1908 made a still more systematic use of dimensions of cells and carefully recorded the linear measurements of all the structures present in the powders and drugs he

examined. These dimensions, however, considered as criteria of purity, are not very satisfactory, since they fail to exclude quite large percentages of adulterants and, moreover, may be indecisive when attempting to establish merely the identity of a powdered drug.

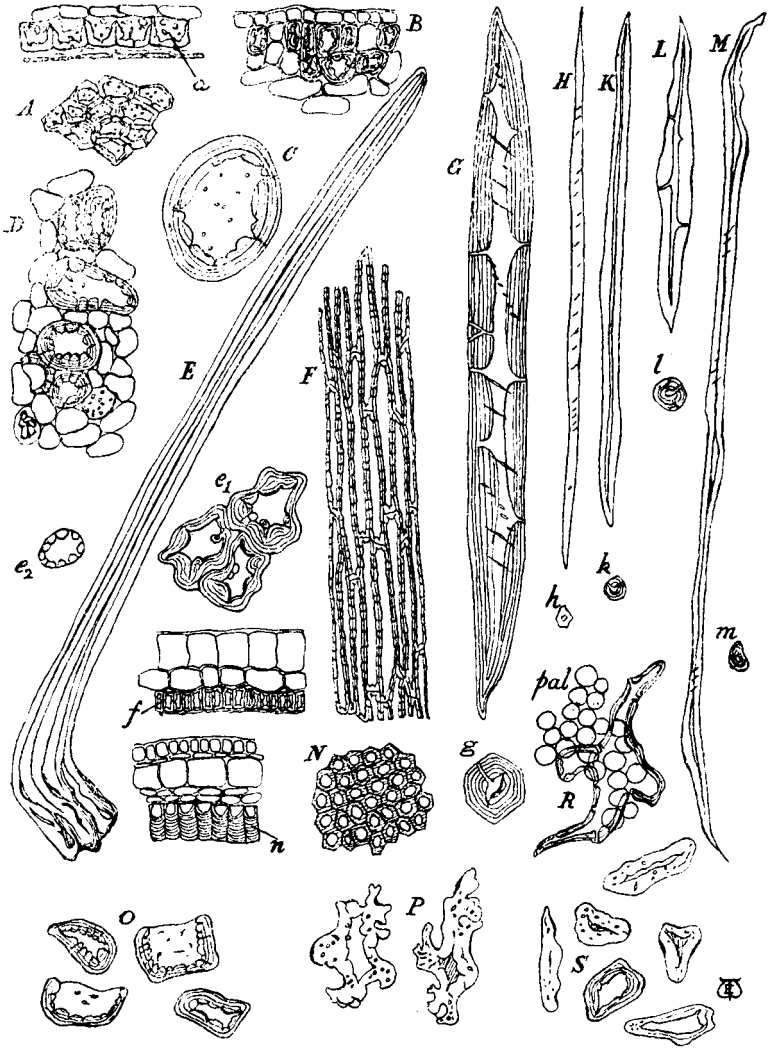

FIG. 1.—Typical Sclereids and Fibres, all × 150. Sclereids of A, beaker-cell layer of *Piper nigrum* Linn., *a*, the same in section; B, hypodermal layer of *Piper nigrum* Linn., C, *Juniperus phœnicea* Linn., D, *Eugenia caryophyllus* (Spreng.) Sprague; E, *Strychnos nux vomica* Linn., e_1, section of trichome bases; e_2, section of limb of trichome; F, *Linum usitatissimum* Linn., f, the same in section; N, *Elettaria cardamomum* Maton var. *minuscula* Burkill, n, the same in section; O, *Cinnamonum zeylanicum* Nees; P, *Viburnum prunifolium* Linn.; R, *Camellia sinensis* (Linn.) O.Ktze. pal, palisade cells; S, *Rhamnus purshianus* D.C.; G, Fibres of *Cinchona succirubra* Pav.; H, *Rhamnus purshianus* D.C.; K, *Cinnamomum zeylanicum* Nees; I, *Sassafras variifolium* (Salisb.) O. Kuntze; M, *Quillaia saponaria* Molina; g, h, k, l, m transverse sections of the corresponding fibres.

SCLEREIDS PER SQUARE MILLIMETRE.

When the cells to be measured form a continuous layer one cell in thickness, it is possible to make an improvement upon simple linear dimensions; this is effected by counting the number of cells per sq.mm. of the layer. The values so obtained provide an automatic averaging of the breadth and length of a very large number of cells, thus yielding data which can replace or supplement linear measurements, the ranges of which frequently overlap so much as to give inconclusive results, when such measurements are used to differentiate between similar tissues derived from closely related plants. The improvement effected has been demonstrated for the sclerenchymatous layer of the testa of various types

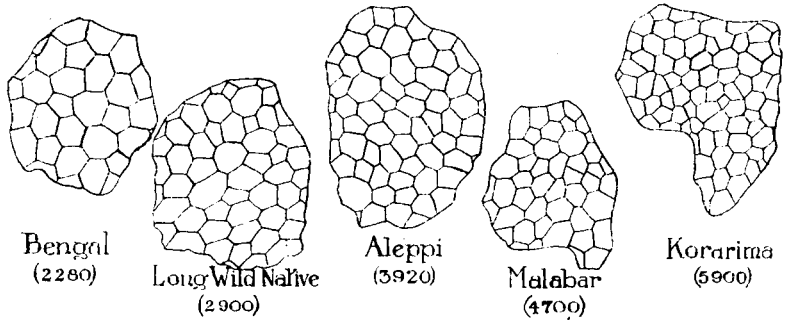

FIG. 2.—Outline of cells in typical fragments of sclerenchymatous layer of cardamom seeds (× 175).

of cardamom seeds (Fairbairn[1]). The drawings in Figure 2 show how difficult it is to make distinctions by the measurements of the diameters of individual cells, but the corresponding numerical values given beneath each piece of sclerenchyma are evidence of the much increased differentiation obtained by their use. These quantities allow eight varieties of cardamoms to be arranged in four distinct groups and, by supplementing the counts with other histological characters, the individual varieties can all be distinguished both in the unground condition and in the form of powder (Fairbairn[2]). This method deserves consideration wherever it is applicable and, in addition to the results for cardamom seeds, values already exist for the sclerenchymatous beaker-cell layer of pepper, viz. 1464 ± 200 beaker-cells per sq.mm. (Wallis and Santra[3]) and for the epidermal sclerenchyma of nux vomica seeds, viz. 570 epidermal sclereids per sq.mm. (Wallis and Fairbairn[4]).

SCLEREIDS PER MILLIGRAMME

Further advances in the use of sclerenchymatous tissues have been made by introducing the concept of mass into the values obtained. When this is done, it becomes possible, not only to identify the materials concerned, but also to assess the purity and to determine the proportions present in a given powder. Where the sclereids are isolated as in French savin, *Juniperus phœnicea* Linn., see Figure 1C (Flück and Haller[5]), or are loosely associated as in clove stalks, see Figure 1D (Wallis and

Santra[6]), and in the hypodermal sclerenchyma of pepper, see Figure 1B (Wallis and Santra[7]) and in the pericycle of ipecacuanha stem (Lupton[8]), the number of sclereids per mg. is easily counted. This is done by using the lycopodium method (Wallis[9]) and staining the material with phloroglucin and hydrochloric acid. The results for the materials quoted are as follows:—

Materials Examined.	Sclereids per mg.
French savin	210
Clove stalks	1,067
Pepper (hypodermal sclereids)	4,585
Black Pepper Husks (hypodermal sclereids)	13,230
Mysore Cardamom seeds (beaker-cells)	9,154
Black Pepper (beaker-cells)	1,500
White Pepper (beaker-cells)	1,619
White Pepper shells (beaker-cells)	10,783
Ipecacuanha stem	33

Measurement of Length per Milligramme

Although the number of epidermal sclereids per mg. of nux vomica seeds has been determined by careful manipulation and found to be 288 per mg. (Wallis and Fairbairn[4]), this value cannot be used as a means of determining nux vomica in powder, because the trichomes obscure the outlines of the cell bases. Each epidermal sclereid is prolonged into a trichome, the limb of which is traversed longitudinally by several (average number 11) narrow lignified strips varying in width from 3 to 10μ, see Figure 1E, e_1 and e_2. In the powder of the seed the strips separate and become broken into small fragments, which have a very characteristic appearance and are easily recognised. It is preferable therefore and comparatively simple to determine the length per mg. of the fragments of lignified rib derived from the trichomes. For this purpose, the powdered nux vomica is mixed with lycopodium and stained with safranin and the lengths of the fragments of rib are measured by using a camera lucida In this way, it is found that there are, on the average, 184 cm. of rib per mg. of air-dry nux vomica (Wallis and Fairbairn[4]).

All these values involving structural units per mg. can be used either to assess the purity of the material concerned or to determine the amount of any one of them in admixture with other substances.

Measurements of Area

Fibres in vegetable materials are more difficult to count than sclereids; this is largely because of their length and the difficulty of deciding how many fibres are represented by the broken portions found in the powders. To obtain satisfactory results with powders of No. 85 fineness, work must, at present, be limited to those materials in which the fibres occur either isolated, as in sassafras bark, or are arranged in single rows, as in cinchona, cassia and cinnamon barks; fibres in bundles cannot be successfully counted in a No. 85 powder. The difficulty of counting the fragments present is best surmounted by finding the total area of the

fragments of fibres present; this is done by tracing the outlines of the fragments with a camera lucida and finding their area by cutting out the tracings and weighing them. In conjunction with the lycopodium method, the area of fibre per g. is determined. This procedure was adopted for the fibres of powdered cinnamon and powdered cassia barks. The number of fibres in the phloem of cinnamon is considerably greater than in the phloem of cassia; moreover, the cork and cortex are removed from cinnamon, but not from cassia and this still further increases the difference between them. The fibres of cinnamon are somewhat more slender, but slightly longer than those of cassia, so that the area of the outlines of individual fibres in the two barks is not very different. The area of fibre-outline per mg. which summates number, length and breadth is therefore markedly greater in cinnamon than in cassia. The values obtained (Saber[10]) are:—

Cinnamon 80 to **85** to 91 sq.cm. per g.
Cassia 11 to **12** to 13 sq.cm. per g.

For these two barks therefore the values are widely separated and can be used, not only to characterise the barks themselves, but also to determine accurately the proportion of either in a mixture of the two or in compound powders.

Area measurements are also used for sclereids which form a layer one cell thick, as in linseed (see Fig. 1 F) (Saber[11]) and in cardamom seeds, see Fig. 1 N, (Fairbairn[1]), the result being expressed as area of the layer per mg. The values found for these seeds are:—

Linseed 28 to **32** to 35 sq. cm. per g.
Cardamom (Mysore) 27 to **28·5** to 29·7 sq. cm. per g.

These values can be used to determine the proportion of linseed in mixed cakes, etc., and of cardamom seeds in mixed spices and compound powders.

Many aërial stems are strengthened to withstand lateral strains by a tubular development of sclerenchyma, often in the pericycle, sometimes in the inner layers of the cortex. The stem bases attached to the rhizomes of *Valeriana officinalis* Linn., show a well-developed cylinder of rectangular sclereids in the inner layer of the cortex and there are similar cells in the bases of the petioles of the same plant. It has been proposed to use these diagnostic elements to determine the proportion of stem and leaf-bases present in powdered valerian rhizome (Flück and Haller[5]). The amount of these sclereids is measured in terms of their area obtained by multiplying together the length and breadth of the particles of sclereid layer seen in the powder. In this way 6·83 sq.cm. of sclereid layer was found to be present in one gram of powdered stem and leaf base. Flück and Haller suggest that the Swiss Pharmacopœia might introduce a standard for powdered valerian rhizome of not more than 0·35 sq.cm. of these sclereids per g., corresponding to just over 5 per cent. of stems in the drug.

Three-Dimensional Masses of Cells

In many substances, sclereids are present in masses, which may be limited in extent and approximately ovoid in shape, as in pimento, or

they may form a dense tissue, as in olive stones. These groups and tissues occur in the powder as three-dimensional particles and it is impossible to count accurately the individual cells in these particles. This difficulty can be overcome either by breaking down the particles into individual cells or by finding some method of calculating the number of cells from the number visible on the upper surfaces of the masses. For breaking down the particles, the oxidising agent used is nitric acid; an important drawback to this method is that the removal of lignin by the oxidising agent modifies or destroys the staining reactions of the cells, thus making it more difficult to identify them in the operation of counting. In attempting to devise a method for making calculations; it is evident that the shape of a mass built up of cells must depend to a large extent upon the shape of the constituent cells. If the cells are chiefly longer than wide, an ovoid mass might be expected, but if they are isodiametric, a subspherical mass would result. For both types of cell aggregate a count of cells along two axes at right angles would give a good estimate of the diameter of the sphere, when the mass is subspherical, or of an imaginary equivalent sphere, when the mass is more or less ovoid (see Fig. 3). Then using the formula for volume of a sphere, viz. $\frac{4}{3}\pi r^3$, the number of cells is calculated. If a sufficient number of particles is used—about 12 to 20—the result of the calculation method agrees with that obtained by disintegration and, since calculation is more rapid and involves no change in staining reaction of the cells, it is to be preferred. Proceeding in this way it has been found (Wallis and Santra[12]) that pimento contains 3546 ($\pm$ 200) sclereids per mg.

Powdered olive stones consist of a certain number of individual cells

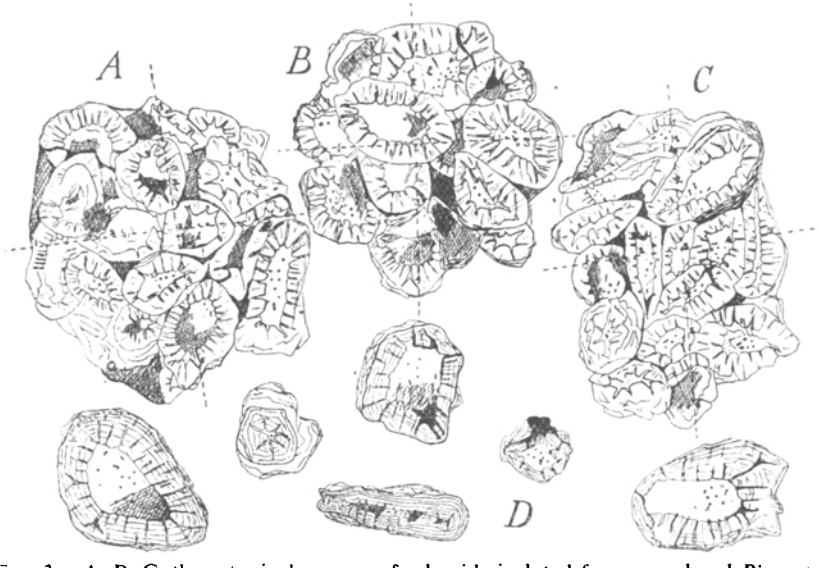

FIG. 3.—A, B, C, three typical masses of sclereids isolated from powdered *Pimento officinalis* Lindl.; D, individual sclereids of various shapes and sizes from powdered pimento. All ×200. The dotted lines are the diameters across which the numbers of cells were counted in calculating the radii of the equivalent spheres.

and broken pieces as well as particles of various shapes consisting of masses of sclereids. The counting of the separated individual sclereids presents no special difficulty, but the particles consisting of masses of cells are much more irregular than the ovoid groups found in pimento. When, however, the same convention of an equivalent sphere is applied to them, it was found that, if at least 12 particles are used, the number of sclereids obtained by calculation agrees with the number found by disintegration. A standard value for olive stones was thus determined, viz:— 15,140 (±900) sclereids per mg. (Wallis and Santra[13]). This value has been successfully applied for the determination of powdered olive stones added to pepper.

Quantitative Distribution of Tissues

By utilising the number of sclereids per mg. of black and white pepper and of the various products (see above) obtained commercially in the grinding of pepper, it has been possible to obtain a quantitative measure of the distribution of the tissues in the fruit of *Piper nigrum* Linn. The values thus established can be used to assess the proportions of the different parts of the fruit, which should be present in commercial products obtained from peppercorns (Wallis and Santra[3]). The quantitative distribution of the tissues in black pepper berries and their products is as follows:—

Black pepper shell in pepper fruit	34·6 per cent.
White pepper shell in pepper fruit	13·9 per cent.
Perisperm in pepper fruit	51·5 per cent.
White pepper shell in white pepper	14·3 per cent.
Perisperm in white pepper (by difference)	85·7 per cent.

Reliability of the Numerical Values

Justification for accepting, as reliable and satisfactory, the general method of working by the use of lycopodium has been provided in connection with many of the experiments. This has been done by making a duplicate and independent determination of the result by a method which did not involve the use of lycopodium. For several commodities the required value can be found by the use of calculations based upon geometrical data derived from measurements of the area or volume or some other character of the unground substance. Whenever this has been done, working with sclereids it has been used for linseed, nux vomica and pepper, the values obtained have always been in remarkably close agreement with those found by the lycopodium method applied to the powder of the same material. Although this type of independent check cannot be made for every powder, the fact that it has confirmed values for a number of materials to which it is applicable gives justification for claiming a similar accuracy for all the materials examined. In this way complete confidence in the results has been established so that, when they happen to differ markedly from results obtained from powdered materials by other methods, the cause of disagreement must

be sought either in some defect inherent in the other method or in some difference in the actual substance examined.

When determining the number of cells per sq.mm. of cardamon seeds the results from the first series of experiments showed a rather large variation, which was greater than was desirable and trials showed that altering the details of manipulation yielded no improvement. It therefore appeared that the variations were probably due to variations in the number of pieces of sclerenchymatous layer used for each value obtained; these varied from 8 to 16 pieces selected at random from a powder of No. 85 fineness. Although a fairly precise estimate of the minimum number of pieces to be used could be made by considering the experimental figures obtained, it seemed desirable to obtain mathematical confirmation of the validity of the deductions made from the experiments. It was, therefore, determined to examine statistically the effect of using different numbers of pieces of the sclerenchyma. For this purpose the mean of the individual results from each of 98 pieces was found and the standard deviation was determined. Curves were then constructed to represent the limits of error above and below the mean that could be obtained by using gradually increasing numbers of pieces of the sclerenchyma. Two curves were constructed, one showing limits for errors in 67 per cent. of the counts and the other in 99 per cent. of the counts. In this way it was shown (Fairbairn[14]) that, when the number of pieces is about 36, the limits of error become fairly constant and for 99 per cent. of the determinations the limit of error is ± 8 per cent. and for 67 per cent. it is ± 3 per cent. This statistical examination creates confidence in the experimental figures based on counts of 36 pieces; it also gives a reliable measure of the amount of variation to be expected and therefore assists in attributing a correct degree of specificity to the values obtained.

Conclusion

A review of these studies of sclereids and fibres, made during the last 15 years, reveals the much extended information which can be gained by applying to them the concepts of number, length, area, volume and mass in addition to the simple observational concepts of form and location. Similar advances have been made in the study of other groups of tissues, but they cannot be discussed in this article.

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