SPICES

Volume II

Morphology · Histology · Chemistry

by

John W. Parry

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Dedicated to my wife
MARY PARRY

PREFACE TO THE SECOND EDITION

The publication of a new edition of this book has afforded an opportunity to add chapters on chervil and tarragon, two herbs that have enjoyed a favored place in European kitchens since ancient Greek and Roman times and that have found increased consumer demand in America in recent years.

It has also afforded an opportunity to add recent information on the composition of the essential oils of the spices as reported in *Chemical Abstracts* of the American Chemical Society and in other scientific journals.

Since almost all of the demand for sweet basil is now met by American growers, the chapter and line drawings of this herb have been revised to cover the domestically cultivated product.

Other additional information has been incorporated into the text, and the glossary has been considerably expanded.

The author would like to take this opportunity to extend his thanks to Sr. Miguel Pascual Giménez, Ingeniero Director, Instituto Nacional de Investigaciones Agronómicas, Ministerio de Agricultura, Murcia, Spain, for information on Spanish paprika; to M. André Darbonne, Syndicat National des Producteurs, Ramasseurs & Collecteurs de Plantes Médicinales, Aromatiques et Industrielles, Milly-La-Forêt, France, for information on chervil and tarragon; to Mr. F. Caligari, Spice Islands Company, California, for his letter on chervil; to Mr. A. W. Pastor, San Jacinto Spice Ranch, Inc., Inglewood, California, for information on the production of sweet basil and tarragon, and for the samples of whole dried leaves of these herbs which he so kindly sent me; and to Mr. Marshall W. Neale, The American Spice Trade Association, New York, for statistical and other information, and for his assistance in obtaining the above samples.

John W. Parry

PREFACE TO THE FIRST EDITION

This work is intended as a short reference book on the structure and chemical composition of spices; to provide helpful information for those whose responsibility it is to examine samples of spices with the microscope; to provide a guide to the microscopy of spices for students of food analysis; to interest students of plant histology and others willing to make a reasonable expenditure of time and study in the structure of spices; to provide information on the structure and chemical composition of spices for the use of all those engaged in, or associated with, the production, manufacture, and distribution of spices; and to convey to the general reader some idea of the external form, the complex but interesting internal structure, and the chemistry of spices.

Part 1 is in no sense an exhaustive enquiry into the anatomy of the parts of plants used as spices, but an attempt to describe as clearly and simply as possible the most important features of the external form and internal structure of the various spices.

Part 2 deals with the chemical composition of spices. The basic chemical composition of spices is much like that of many other plants. However, their volatile oils, oleoresins, pigments, glucosides, and, in some, fixed oils, are chemical compounds of special interest. It is these that make the spices so desirable as food-flavoring agents. These substances have been a matter for investigation by a great number of people over a very long period of time, and are still the subject of enquiry by many capable chemists. Much is known, and much is yet to be learned, about the complex organic chemical compounds contained within the spices. No attempt has been made to discuss the chemistry of the numerous complex chemical compounds involved. Such information can be found in larger reference books, abstracts, and papers.

Part 3 consists of photomicrographs of the spices.

For information on the production of spices the author expresses his gratitude to the Director of Agriculture, Kuala Lampur, Malaya Union; Director of Agriculture, Department of Agriculture, Peradeniya, Ceylon; Director of Commerce, Department of Commerce, Ceylon; Director of Agriculture,

Department of Agriculture, Chepauk, Madras, India; Director of Agriculture, Department of Agriculture, Zanzibar, East Africa; Director of Agriculture, Department of Agriculture, Sierra Leone, Africa; Director General, Ministério da Economia, Lisbon, Portugal; Grenada Co-operative Nutmeg Association, Grenada, British West Indies; to Mrs. Ezra Winter, House of Herbs Inc., Salisbury, Connecticut, for samples of peppermint and spearmint; and to Mr. Marshall W. Neale, Director of Information, American Spice Trade Association, New York, for his assistance in obtaining these samples.

John W. Parry

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Part 1

MORPHOLOGY AND HISTOLOGY

INTRODUCTION

Morphology

Morphology is the study of external organic form. In this work, it is the study of the external form of the parts of plants employed to give flavor, relish, and piquancy to foods and found as such in the products of the spice merchant.

It must be kept in mind that spices are *dried* products. The size, shape, color, and general appearance of any one of them will vary to some extent from similar properties in their fresh, living counterparts. Should any descriptive term not be understood it will be found explained in the glossary.

Histology

By histology is meant here the microscopic structure or anatomy of the spices and a study of the cells and tissues of the parts of plants used as spices.

Such a study involves the use of a microscope and other equipment. The microscope is an important, indispensable item. Many fine types are manufactured today. One may buy a very high quality microscope equipped with an incorporated light source, aplanatic condenser, mechanical stage, apochromatic objectives, quadruple nosepiece, compensating oculars, and other advanced features.

However, such an elaborate and expensive instrument is not required for our purpose. An inexpensive microscope equipped with coarse and fine adjustment, a $\times 10$ ocular, two objectives, $\times 10$ and $\times 25$, and substage condenser, will do most of the work. A little more money will buy a very serviceable microscope equipped with a regular Huygenian $\times 10$ ocular, a revolving triple nosepiece, three achromatic objectives, coarse and fine adjustment, a revolving stage, and an Abbe condenser, which will give excellent results. The three objectives recommended are $\times 10$, $\times 25$, and $\times 50$. Very high power is not required in a study of the tissues and cells of plants.

For cutting sections a microtome is a very efficient precision instrument,

but satisfactory sections can be cut with a flat-sided razor-type knife obtainable from any laboratory supply house at small expense, or with a safety razor blade. The safety razor blade is a very useful instrument, especially in obtaining sections of very small fruits or seeds.

Inexpensive polarizing apparatus can be easily and quickly made by cutting two discs from a small sheet of Polaroid, one large enough to fit in the filter ring below the substage condenser, or to be otherwise mounted in that position, to act as the polarizer; and one small enough to lie on top of the ocular, to act as the analyzer. The disc to be used as the analyzer can be fitted into a bored cap made from the end of a small, cylindrical paper or plastic box of suitable diameter. This is a useful arrangement and permits the easy rotation of the analyzer. Polarized light is a helpful aid in the study and identification of starch, crystals, and lignified tissues.

Measurements can be made with an ocular micrometer. Such a micrometer consists of a glass disc on which is engraved a scale of divisions, usually 50, without value. The disc is laid upon the diaphragm within the ocular, scale side downward. To give a value to the divisions of the ocular micrometer they must be calibrated with those of a stage micrometer. The scale of the stage micrometer is engraved upon a glass slide and usually consists of 100 divisions, each with a value of 0.01 mm. or 10 microns. The stage micrometer is brought into focus and the number of divisions covered by the entire ocular micrometer scale is noted, multiplied by 10 and divided by 50. This will give the value in microns of each division of the ocular micrometer. For example, let us say that the entire micrometer scale covers 69 divisions of the stage micrometer scale, then 69 $\times$ 0.01 mm equals 0.69 mm, and 0.69 mm $\times$ 1000 equals 690 microns (or simply 69 $\times$ 10 equals 690), and 690 microns divided by 50 equals 13.8 microns to each division of the ocular micrometer scale.

Calibrations must be made for each combination of ocular and objective. The higher the power the less the number of microns to each division of the ocular micrometer scale. Glass ocular and stage micrometers are inexpensive and obtainable from any laboratory supply house.

Dissecting needles can be obtained from a laboratory supply house, or they can be made by attaching ordinary sewing or darning needles to wooden handles. A bent needle can be obtained by heating a sewing or darning needle in a flame until it is red hot, bending, reheating, and tempering in cold water. Dissecting needles are useful for pulling apart fibrous tissues.

A pair of fine-pointed forceps will be required, and a pair of small scissors should be at hand.

A number of glass slides and cover glasses will be required; square cover

glasses are recommended. Water can be contained in any small table glass. Medicine droppers are excellent for conveying liquids to slides.

A spice may be soaked in water contained in a small glass, but if it requires boiling then a pyrex beaker must be used. A beaker of pyrex glass may be safely employed over a bunsen burner, on a hot plate, or on a kitchen stove; a useful size is 50 ml.

A supply of household bleach, concentrated sulfuric acid, iodine-potassium iodide solution, and chlor-zinc-iodine will suffice for reagents and stains. If desired, a supply of phloroglucinol and hydrochloric acid may be included. Phloroglucinol ((5% in 90% alcohol) followed by hydrochloric acid diluted with an equal volume of water, is a useful stain for confirming the presence of lignified tissue. Lignified cell walls stain red or reddish-violet with phloroglucinol.

A hand magnifying glass is useful, or better still, the type of magnifier used by watchmakers. This is attached to a narrow, spring-like metal band which encircles the head and holds the lens in front of the eye. With its use both hands are free to section or dissect the spice.

It is the ground spice that is usually examined under the microscope. But before the analyst can identify a spice under examination he must be familiar with the tissues characteristic of that spice. A study of sections of whole spices will enable the analyst to identify the fragments of tissues of ground spices seen in the microscope field. Failing the knowledge obtained from such a study he must have reference to this or other texts on the microscopical structure of spices.

It is nearly always necessary to soak the spice in water to soften it sufficiently for sectioning. Some spices are very hard and must be softened by boiling in water; a little sodium hydroxide or a pinch or two of washing soda (sodium carbonate) added to the water will assist the process.

After the spice is soft enough to section, it should be held firmly between the forefinger and thumb, or held firmly against the surface of a glass slide laid upon the work table, and the desired section cut with a cutting knife or razor blade. If the spice cannot be held firmly with the hand it may be embedded in paraffin wax. This will provide support and leave no foreign material clinging to the section. If the wax does not fall away cleanly it can be removed by immersing the section in hot water. A transverse (cross) section is obtained by cutting across the longitudinal axis, a longitudinal section by cutting through the longitudinal axis, and a tangential section by cutting parallel to the longitudinal axis but not through the center of the spice.

The section is placed in a drop or two of water on a glass slide and covered with a cover glass. If the edge of the cover glass is brought into contact with

the water and the glass lowered gently with the aid of a dissecting needle, no air bubbles will be left between the cover glass and the slide. The section may then be examined with the low power of the microscope. Higher powers can be used to permit a study of the cells, cell inclusions, and tissues in greater detail.

Seen at this time, the cells will be partly expanded as a result of the soaking process, and the cell inclusions will be visible. The pigments may be decolored and the cells further expanded by treatment with dilute household bleach. Sections may be bleached on the slide by drawing the bleaching solution under the cover glass. This is done by applying a piece of absorbent paper tissue or blotting paper to one edge of the cover glass and the bleaching reagent to the other. The water is drawn off and replaced by the bleach. Many bubbles arise in the process of bleaching but most of these are removed when the bleach is replaced with water after the section is decolored. The few bubbles remaining will not interfere with the study of the subject. If it is now desired to stain the section, chlor-zinc-iodine is drawn under the cover glass. Cuticle, cutinized, and lignified cell walls will stain yellow; cellulose walls, blue; starch, dark blue; proteins, brown. Regarding lignified cells it should be said that if sections are left too long in the bleaching solution, all or part of the lignin may be dissolved and the walls will give a blue color with chlor-zinc-iodine (cellulose).

Unbleached sections may be stained by treatment with iodine-potassium iodide solution followed by chlor-zinc-iodine. Color reactions will be similar to those given above except for such interference as pigments present. The presence of lignified tissues may be confirmed by staining another section with phloroglucinol.

Iodine-potassium iodide solution stains starch dark blue; aleurone, yellow to brown. It should be remembered that boiling, and solutions of alkali hydroxides, cause swelling and destruction of starch granules. For a microscopic study of starch the spice should be softened in cold or warm water, sectioned, and examined in water mount without further treatment. The presence of starch can be confirmed by drawing iodine-potassium iodide solution under the cover glass.

Concentrated sulfuric acid dissolves cellulose quickly; chars and blackens lignin, but does not alter cuticle or cutinized cell walls. Suberized cell walls are also unaffected by concentrated sulphuric acid.

Ground spices may be mounted directly in a drop or two of water placed on a glass slide, overlaid with a cover glass, and examined under the microscope. Ground spices are usually of requisite fineness for direct examination but if the fragments appear too coarse or too densely packed they may be

rubbed out by applying gentle pressure to the cover glass and making a to-and-fro motion. A pencil with a clean eraser on the end is useful for this purpose. There is enough friction between eraser and glass to permit the movement of the cover glass to-and-fro without messing it up as occurs when using the finger, necessitating the removal, cleaning, and replacing of the cover glass. Bleaching and staining, if required, may be carried out in the manner described above.

Tissues will now be discussed. The simple tissues are parenchyma, collenchyma, and sclerenchyma. Parenchyma tissue is the most common and consists of cells with thin or moderately thick cellulose walls, with or without intercellular spaces. Parenchyma cells in living plants are approximately isodiametric, but in the dried spices they are nearly always collapsed and compressed, and even when expanded with the aid of reagents may fall short of their original shape, frequently appearing irregular or elongated. Parenchyma cells occur in all of the spices; they are abundant in the mesocarp of fruits, the mesophyll of leaves, and in cortex tissue. Epidermal cells are modified parenchmya cells, frequently polygonal in shape in surface view, rectangular or oblong-rectangular in cross section.

Collenchyma is a supporting tissue, and consists of cells with irregularly thickened walls or walls thickened at the corners of the cells. The walls are composed of cellulose and are highly refractive, appearing bright and shiny; they stain blue with clor-zinc-iodine. Collenchyma occurs in the pedicels of the spice fruits of the Umbelliferae except cumin; in the leaves, petioles, and stems of the spice herbs of the Labiatae; and in some other spices.

Sclerenchyma is a supporting, strengthening and protective tissue consisting of cells with thick, lignified walls. Sclerenchyma occurs in many forms. The chief kinds of sclerenchyma cells are stone cells and sclerenchyma fibers. Stone cells vary greatly in size and shape, but are not much longer than broad and have very thick, pitted walls. Sclerenchyma fibers are elongated cells, usually with pointed or tapering ends, and with thick, usually pitted, walls. Sclerenchyma is present to some extent in nearly all of the spices.

Complex tissues are phloem and xylem. They form the conducting or vascular tissue of the plant. In the widest sense, phloem includes sieve tubes, companion cells, parenchyma, and fibers. The parenchyma is known as phloem parenchyma, and the fibers as phloem or bast fibers. Xylem includes vessels, parenchyma, and fibers. The parenchyma is known as xylem parenchyma, and the fibers as xylem or wood fibers. Fibers are not always present in phloem and xylem. In the text, the term *vascular bundle* is used to indicate a bundle of phloem and xylem without fibers, and the older term *fibrovascular bundle* to indicate a bundle of phloem and xylem with fibers. A vessel is a

tubelike series of cells characterized by lignified thickening deposited in various ways on the inside of the walls. The degree of lignification varies. Vessels are known as annular, spiral, scalariform, reticulated, or pitted. In annular vessels the thickening is laid down in rings; in spiral vessels in the form of spiral coils; in scalariform in transverse bars; in reticulated vessels in the form of an irregular network; in pitted vessels the thickening is nearly uniform except for small areas or pits.

The drawings and photomicrographs appearing in the text are by the author. Space does not allow all the descriptive drawings that could be made, nor the inclusion of all the photomicrographs which are on hand, to illustrate the histological structure of the spices, but it is hoped that those that appear in this work will be of assistance to the analyst, investigator, or student, and of interest to the general reader.

I. ALLSPICE

Pimenta officinalis Lindl
Family: *Myrtaceae*

[ALLSPICE, PIMENTO, JAMAICA PIMENTO, JAMAICA PEPPER, CLOVE PEPPER]

GENERAL

An evergreen tree, 25 to 30 feet in height; trunk erect, bark gray; much branched; round-topped; foliage dense. Leaves opposite, oval-oblong to elliptical, entire, glandular-punctate on lower surface; 4 to 6 inches in length; deep green, lustrous. Flowers borne on racemose cymes, small, white to greenish-white, sepals 4, petals 4, stamens numerous; ovary 2-loculed, inferior.

Allspice fruits are harvested when mature but still green. The fruits are dried in the sun; their color changes during drying to dark reddish-brown.

Allspice trees bear fruits in about 7 years and are productive for about a further 12 years.

MORPHOLOGY

A dried, dark reddish-brown, hard berry with a rough surface caused by numerous protuberant oil cavities underlying the exocarp. Calyx and style remains at the apex, stalk scar at the base or, occasionally, a short stalk or portion of stalk. In shape, nearly globular. In size, from about 4 to 7 mm. Pericarp woody, brittle, and around 1 mm in thickness.

The berry has 2 locules separated by a thin dissepiment or partition. Each locule contains a single, reniform, hard, dark brown seed. The embryo is large, curled, with prominent radicle and small cotyledons.

The aroma is fragrant, clove-like; the taste is clove-like, strongly aromatic, pungent.

The name "allspice" is given to this spice because its flavor is said to resemble a combination of cinnamon, nutmeg and cloves.

HISTOLOGY

Pericarp

This consists of exocarp, mesocarp, and endocarp.

Exocarp. In cross section, consisting of epidermis with stomata and short, thick-walled, unicellular, nonglandular hairs up to about 150 microns in length. These hairs are easily broken off in the preparation of the section and are best studied in surface view.

The cells of the epidermis are small, up to about 10 microns, long axis; tangentially oblong-rectangular or nearly so, and have strongly cutinized outer walls. In surface view the epidermal cells are small, from about 5 to 22 microns, and polygonal in shape.

Mesocarp. Cross section. In the outer mesocarp, near the epidermis, there is an oil zone consisting of numerous cavities in which the essential oil of allspice is secreted. These cavities are oval or round in shape. The oval cavities vary in size to about 210 microns, long axis, and to about 150 microns, short axis. The round cavities vary in size from about 60 to 210 microns in diameter; the majority are from about 150 to 180 microns in diameter.

Scattered throughout the mesocarp are numerous pitted, striated stone cells varying greatly in amount of wall thickening, in size and shape. They occur singly, in small groups, and in masses. Individual cells reach up to about 110 microns, long axis, and in the apex region of the mesocarp to about 150 microns; the latter stone cells have, in general, thinner walls.

Fibrovascular bundles occur in the mesocarp. Seen in longitudinal section the vessels are spiral; the fibers have thick, pitted walls, and vary in width and length. The fibers are not numerous.

The ground tissue of the mesocarp consists of thin-walled parenchyma cells, somewhat compressed, and irregular in shape. Rosette crystals of calcium oxalate are scattered throughout the mesocarp but occur more abundantly near and between the oil cavities.

Groups of stone cells occur in the inner mesocarp. These cells vary in size from about 30 to 60 microns, long axis, and the groups extend to about 1300 microns in length. A few layers of compressed parenchyma cells separate the inner mesocarp stone cells from the endocarp.

Endocarp. The endocarp consists for the most part of a layer of compressed parenchyma tissue. In the apex region, near the locule partition in the area of the calyx remains, the endocarp cells become rectangular in shape, and average about 12 microns square. In surface view, for the most part, the endocarp cells are elongated, narrow, and thin-walled.

Locule Partition

The locule partition or dissepiment consists of parenchyma tissue with a few stone cells and numerous crystals of calcium oxalate; both rosette and prismatic crystals occur. Vascular tissue traverses the parenchyma. A very thin cuticle is present on both surfaces.

Seed

Seed Coat. In cross section, the seed coat consists of compressed parenchyma tissue contained between an outer and inner epidermis except on the outer convex and inner flat sides of the seed where the outer and inner epidermis enclose several layers of cells filled with a rich orange-red, gummy or resinous substance. These cells have been called "port wine" cells by early writers and are frequently referred to as such nowadays. This description might apply, but only approximately so, when the resin cells are clumped together and less penetrable to transmitted light. The resin cells are an important identifying characteristic of ground allspice.

The outer walls of the outer epidermis are strongly cutinized, those of the inner epidermis weakly so. In surface view the cells of both the outer and inner epidermis are elongated, narrow, and have faintly beaded walls. They vary in arrangement, with a tendency to parquetry in places.

Vascular tissue with delicate spiral vessels occurs in the seed coat and is usually seen on the inner side of the resin parenchyma adjoining the inner epidermis.

Embryo. The embryo is curled and consists of a large radicle and two small cotyledons. In cross section, the epidermis consists of small, clear cells covered by a very thin cuticle. Underlying the epidermis there is an oil zone with cavities smaller than, but similar to, those of the pericarp. Rudimentary vascular tissue occurs in the central zone of the embryo; delicate spiral vessels are observed in longitudinal section. The remaining tissue consists of small, isodiametric parenchyma cells containing mostly round but some truncated granules of starch, 4 to 12 microns in diameter, and with central hilum.

Pedicel. An untreated cross section of the pedicel viewed under low power shows the epidermis and cortex tissue dark reddish-brown; phloem tissue and pith dark orange; fibers and xylem obscurely yellow, the fibers masked by the

surrounding orange color, and the xylem by light transmitted through the lumen of the radial rows of vessels and accompanying tissues.

For a detailed examination, a section should be decolored and the cells expanded by treatment with bleaching solution. A second section should be so treated and then stained with chlor-zinc-iodine.

In cross section, the pedicel consists of (a) epidermis of small cells with strongly cutinized radial and outer walls; (b) cortex of thick-walled, irregular-shaped cells varying in size to about 67 microns, long axis; intercellular spaces; rosette crystals of calcium oxalate occurring in some cells; and occasional oil cavities, round, oval or elliptical, from about 65 to 85 microns, diameter or long axis; (c) a broken ring of thick-walled pericycle fibers; (d) thick-walled phloem tissue with some cells containing prismatic crystals of calcium oxalate; (e) an unbroken ring of xylem tissue with vessels in radial rows, fibers, parenchyma cells, and xylem ray cells; (f) pith of thick-walled parenchyma cells, some containing rosette crystals of calcium oxalate, and thick-walled fibers singly or in groups.

In surface view, the epidermis consists of rectangular to polygonal (mostly the latter), thick-walled, frequently pitted or beaded cells up to about 25 microns, long axis; numerous hairs, straight or curved, pointed, unicellular, varying in length to about 165 microns. No stomata.

Seen in longitudinal section, the pericycle fibers are long, thick-walled, pitted, with lumen narrow to wide. The pith fibers are shorter than those of the pericycle, pitted, and with narrow lumen. Noteworthy in the phloem tissue are chains of prismatic crystals of calcium oxalate; crystals imperfect, but of tetragonal prism-pyramid, rhombic, and cubic types up to 12 microns, long axis. The xylem consists of numerous spiral vessels, fibers, and thick-walled, pitted parenchyma cells.

Ground Allspice

The most important identifying characteristics of ground allspice are (a) the so-called port wine cells of the seed coat, the resin cells; (b) the stone cells, which are numerous; (c) fragments of the pericarp with oil cavities; (d) fragments of the exocarp, and short unicellular hairs; (e) starch; and (f) fragments of the embryo.

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