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Species identification of nonwood plant fibers

1. Scope

The fibrous elements of the nonwood plant species, which are commonly encountered in papermaking or that are expected to have the potential of being used for this purpose, may be identified on the basis of their morphology as revealed by the microscope. The purpose of this method is to provide some of the details, which are useful in making an identification of an unknown nonwood plant specimen. This method can be used whether a coarse undefibered specimen is present or samples of pulp, paper or other paper products are provided.

2. Apparatus

The only special equipment required for this method is a compound microscope equipped with a calibrated micrometer eyepiece.

3. Significance

To assist an analyst in determining what type of commercially available nonwood fiber is present either in the rough form or present in a pulp, paper or other paper product.

4. Reagents

The majority of the specimens depicted in this method were delignified with an acidified sodium chlorite solution. The required reagents are: 50% acetic acid (CH_3COOH), sodium chlorite (NaClO_2), 1.0% NaOH, 0.5N HCl, and Graff's C-Stain or Sellegger's stain. A second, and more rapid, procedure requires: 1% sodium hydroxide (NaOH) solution and 1% methylene blue solution. The solutions need only be of the approximate strengths indicated. As an additional reference, TAPPI T 401 "Fiber Analysis of Paper and Paperboard" for information on Graff's C-Stain and Sellegger's stain. 10% sodium hydroxide (NaOH) solution can be used to defiber specimens of reference fibers.

Approved by the Standard Specific Interest Group for this Standard Practice



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5. Sample preparation

Caution: Use safe laboratory practices in handling hot chemicals. The sodium chlorite procedure should be handled with extreme care. If the reaction is carried out too rapidly this mixture could react violently. Use of a vented fume hood with sash and a personal protection face shield are necessary.

- 5.1 A fibrous suspension may be prepared by the acidified sodium chlorite procedure as follows:
 - 5.1.1 Place 1 g of a stalk or coarse specimen in a 170 x 20 mm test tube and wet with 30 mL of H₂O.
 - 5.1.2 Add 5 g of solid NaClO₂.
 - 5.1.3 Add 10 mL of 50% CH₃COOH at 60°C and lower test tube into a water bath heated to 60°C.
 - 5.1.4 Lightly swirl the contents, stopper, and allow to react for 1 h.
 - 5.1.5 At the end of 1 h, stir contents with a glass rod and let stand for 24 h at 60°C. The stoppers should be kept in place to prevent the loss of reaction liquor.
 - 5.1.6 At the end of the 24 h period, drain off the reaction liquor, wash the mat three times in distilled water, and shake vigorously to insure uniform fiber separation.
 - 5.1.7 The 24 h reaction period, steps 2 to 6, may have to be repeated as many as three times depending upon the ease of fiber separation of the material.
 - 5.1.8 If the fiber mat was removed for washing, place it back in the test tube. Add 10 mL of 1.0% NaOH, heat to 60°C and hold for 1 h, drain liquor, macerate, and wash pad in water three times.
 - 5.1.9 Treat the fibrous pad with 10 mL of 0.5N HCl for 0.5 h at 20°C and wash three times.
 - 5.1.10 Place a few drops of the suspension on a microscope slide, stain in the normal manner with C-Stain or Selleger's stain, cover, and examine. (See TAPPI T 401 for proper staining technique.)
- 5.2 The rapid hot alkali procedure may alternatively be employed as follows:
 - 5.2.1 A 1 g sample of the specimen pulp or paper is boiled for a few minutes in a 1% NaOH solution.
 - 5.2.2 Wash the sample with water and shake to insure complete separation.
 - 5.2.3 Place a few drops of the fibrous suspension on a microscope slide, stain with a drop of methylene blue, Graff's "C" stain or Selleger's stain solution, cover, and examine.

NOTE 1: The 1941 and 1947 versions of this method contained only the rapid procedure detailed in section 5.2. The more rigorous procedure detailed in 5.1 was added in the 1978 revision of the method. Analysts are referred to Reference 5 and 6 for further information on the use of the stains specified in this method. Depending on the application, analysts may find other stains useful in the identification of fibers.

6. Reference standards

When authentic specimens of commercial fibrous materials are available, it is highly desirable that known fibrous suspensions be prepared for purposes of comparison with tentatively identified samples. A coarse material may be defibered by boiling a small amount of the commercial fiber in 10% NaOH solution for approximately 30 min, followed by washing and vigorous shaking in water. A pulp or paper reference can be defibered and prepared using 5.18 through 5.1.10.

7. Procedure

7.1 A thorough microscopical examination of the fibrous suspension includes an enumeration of the cell types present, their characteristic markings, and a measurement of cell dimensions. The micrographs included in this method have been prepared mostly at the same magnification to facilitate direct comparisons. A tentative identification may be made through the use of the dichotomous key in Table 1. In using the key, select from the pair numbered 1 the description that best fits the material under consideration. If a number is found at the right-hand end of the line, refer next to the pair of descriptions bearing that number and repeat the procedure. By this method of selection, a description will ultimately refer to a species, or group of species, which possesses the required characteristics. Analysts should keep in mind that not all non-woody fibers can be covered in this method. Unknowns will be encountered.

7.2 Reference should next be made to the description, fiber dimensions, and the photomicrographs of this particular species as a further check upon the identification. A final comparison of the unknown with authentic material as previously mentioned should provide conclusive proof as to the identity of the sample.

Table 1. Species identification of nonwood plant fibers; key to nonwood plant papermaking fibers

1. Fibers long, averaging more than 15 mm in length; complete fibers rarely seen under an ordinary microscope	2
1. Fibers comparatively short, averaging less than 10 mm in length, complete fibers frequently seen by moving the glass slide about the stage of the microscope	5
2. Fiber flat, ribbonlike; usually twisted about its longitudinal axis	Cotton
2. Fiber cylindrical, not twisted; prominent transverse fractures in the cell wall	3
3. Individual fibers variable in width, up to 80 μm in width at their broadest portions	Ramie
3. Individual fibers quite uniform in diameter; the broadest fibers not more than 50 μm wide	4
4. Narrow lumen clearly defined within thick fiber walls; fibers pointed at their ends	Flax
4. Cell cavity rather obscure but often wider than the fiber wall; fiber ends blunt	Hemp
5. Fibers isolated or accompanied by occasional cells of other types	6
5. Vessel segments and parenchyma cells abundant together with fibers	9
6. Fiber unusually variable in diameter, a central segment averaging about twice as wide as the rest of the fiber	Mitsumata
6. Diameter of fiber relatively uniform throughout nearly its entire length	7
7. Fiber lumen irregularly constructed at intervals to an extremely narrow canal	Jute
7. Fiber lumen uniform in diameter	8
8. Fiber lumen broad, distinct; cell walls thin to moderately thick	Manila hemp
8. Fiber lumen narrow; often indistinct; cell walls thick	New Zealand flax
9. Individual epidermal cells or fragments of epidermal tissue abundant; margins of these cells rather distinctly toothed	10
9. Epidermal cells infrequent; their margin undulating rather than distinctly toothed	12
10. Parenchyma cells relatively narrow, none present more than 20.5 μm wide, epidermal cells narrow (less than 14 μm) accompanied by numerous erect trichomas, hooked at their apices	Esparto
10. Parenchyma cells narrow to barrel-shaped; up to 130 μm in width; epidermal cells usually greater than 14 μm in width occur together with straight trichomes, the latter appressed to the surface of epidermal cells or comparatively sparse and erect	11
11. Trichomes in conspicuous and erect; dermal cells as fattened, spurlike projections; epidermal cells profusely pitted; stomata of the epidermis accompanied by dentate guard cells; fiber diameters 5.1 to 13.6 μm	Rice
11. Trichomes in conspicuous and erect; epidermal cells rarely pitted; guard cells surrounding the stomata with entire margins; fiber diameters 6.8 to 23.8 μm	Barley, oat, rye, wheat
12. Fibers relatively long (up to 4.3 mm in length; averaging 2.7 mm) accompanied by thin-walled, ribbon-shaped fibers up to 40 μm in width	Bamboo
12. Fibers relatively short (up to 2.9 mm in length; broadest fibers not ribbon-shaped (34 μm wide)	13
13. Vessel segments long (1350 μm); maximum fiber diameter 34 μm , parenchyma cells as long as 850 μm	Sugar Cane
13. Vessel segments comparatively short (600 μm maximum); maximum fiber diameter 24 μm , parenchyma cells as long as 325 μm	Corn

8. Structure of the plant stem

8.1 Although the pulping process so destroys the original tissue of the plant stem that the individual cells are more or less completely separated and appear as isolated units, a general introduction to the structure of the plant is essential to an understanding of the cell types to be described. The true fiber of the nonwood plant, which is the only cell type contributing in any degree to the strength of the paper, is accompanied by other types of cells which frequently are important aids in identification.

8.2 The plants from which the commercial fibers described herein are obtained may be divided into two general classes according to their botanical characteristics: monocotyledons and dicotyledons. The monocotyledons are the plants having parallel-veined leaves such as the grasses, lilies, and palms. The dicotyledons have net-veined leaves and include such plants as kenaf, hemp, okra, flax, and the common broadleaf trees. The two classes may be separated by an examination of the internal structure of the plant stem.

8.3 The transport of water and food through the plant is accomplished in both monocotyledons and dicotyledons by a system of cells arranged in a long series known as sieve tubes and vessels. The thin walls, frequently modified by openings known as sieve areas and pits, together with the large lumens of these cells, are commonly explained on the basis of their evolutionary adaptation for the conduction of liquids. As though to compensate for these weak conducting cells, they are accompanied by another cell type, the fiber, which contributes strength and rigidity to the

plant. Fibers are long, slender cells characterized by relatively thick, infrequently pitted walls and a narrow cell cavity or lumen.

8.4 The arrangement of the conducting or vascular tissue in the plant stem is characteristically different in the monocotyledons and the dicotyledons. In the former group the conducting tissue and the accompanying fibers are arranged in vascular bundles which are generally distributed at random throughout the greater part of the plant stem (Fig. 1). The vascular tissue of the dicotyledons, conversely, are arranged in a definite ring pattern, or frequently an unbroken ring of vascular tissue without distinct bundles may serve for purposes of conduction and mechanical support (Fig. 2). The inner portion of this ring of vascular tissue is the xylem, composed chiefly of xylem fibers and vessels. Just outside of the xylem is a narrow layer of living meristematic cells known as the vascular cambium and beyond this lies the phloem, consisting for the most part of sieve tube elements, parenchyma, and phloem fibers. Surrounding the vascular tissue are successive protective layers known as the pericycle, cortex, and epidermis. The fibers used in papermaking may be vascular bundle fibers from the stem or leaf of the monocotyledons, or, if derived from the dicotyledons, they may be from the phloem, pericycle, cortex, or xylem of the stem. The dicotyledonous phloem fibers are commonly designated as bast fibers.

8.5 The cotton fiber illustrates still another source of commercial fiber. This is the seedhair, a cell that originally was attached to the seed of the plant. In contrast to the structural fibers, which are separated only by disintegration of the plant tissue which they constitute, the seedhairs are individual units in nature.

8.6 The fibers to be described in the ensuing paragraphs may be classified in the following manner:

8.6.1 Structural or stem fibers

8.6.1.1 Vascular bundle fibers - monocotyledons (Gramineae): reed, *arundo donax*; oat, *Avena barbata*; barley, *Hordeum sp.*; rice, *Oryza sp.*; elephant grass, Napier grass, *Pennisetum purpureum*; reed, *Phragmites communis*; rye, *Secale sp.*; Columbum grass, *Sorghum alum*; broomcorn, *Sorghum bicolor*; Piper sudan, *Sorghum sudanense*; esparto, *Stipa tenacissima*; triticale; wheat, *Triticum sp.*; emmer, Russian Wheat, *Triticum dicoccum*; corn, *Zea mays*; bamboo, *Phyllostachys bambusoides*; sugar cane, *Saccharum officinarum*.

8.6.1.2 Bast or bark fibers - dicotyledons: ramie, *Boehmeria nivea* (Urticaceae); hemp, *Cannabis sativa* (Moraceae); Sunn hemp, *Crotalaria juncea* (Fabaceae); cottonstalk, *Gossypium herbeceum* (Malvaceae); kenaf, *Hibiscus cannabinus* (Malvaceae); okra, *Hibiscus esculentus* (Malvaceae); flax, *Linum usitatissimum* (Linaceae).

8.6.1.3 Seedhair fibers: cotton, *Gossypium sp.* (Malvaceae).

8.6.1.4 Leaf fibers: sisal *Agave sisalina* (Amaryllidaceae); Manila hemp, Abaca, *Musa textilis* (Musaceae).

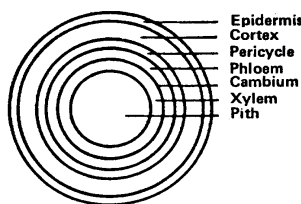


Fig 1. Dicotyledonous stem.

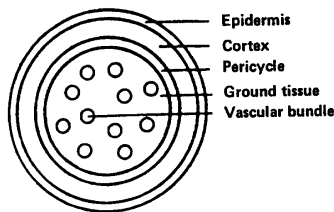


Fig. 2 Monocotyledonous stem.

9. Cell types

9.1 Before proceeding with detailed descriptions of pulp or paper samples from these species, the general features of each type of cell will be outlined.

9.2 Fibers are long, relatively narrow, empty cells with typically thick, usually lignified walls. Most commonly the cell ends are pointed. Pitting is not a characteristic feature of fiber walls, but when present, the pits will appear as simple rounded openings or slits.

9.3 Vessels consist of a series of elements lying end to end to form conducting tubes in the xylem. These cells include large empty cell cavities, and their breadth is greater than that of the fibers. The ends of vessel elements are commonly blunt and, unlike the fiber, the end wall is perforated to permit more ready communication between the individual cells. Vessel walls are usually thin and characteristically marked by numerous pits of various shapes. Annular vessels, which consist of a series of rings, and spiral vessels, the cell wall of which consists of a spiral winding, occur less frequently.

9.4 Parenchyma cells remain alive for a longer period in the plant and consequently retain active protoplasm in the cell cavity. Cells of this type are thin-walled and sparingly pitted; their end walls are complete (not perforated) and more or less right-angled, and their length is often not much greater than their width.

9.5 Epidermal cells are derived from the tissue that forms the outermost layer of the leaf or stem. Their most distinctive feature is a wavy or toothed cell margin. When not completely separated one from another, the cells are clearly shown to interlock. Stomata, openings to provide for the exchange of gases between the plant and the atmosphere, are interspersed over the plant epidermis. Certain cells of the epidermis are variously altered in shape to short spur like projections and even to long tubular hairs. Such cells are known as trichomes, and in as much as the identification of a sample may hinge upon their presence or absence, the necessity for accurate observation and interpretation in microscopic work is apparent.

10. Characteristics of individual species

10.1 *The grasses, Gramineae.* Included in the grass family are sugar cane, the cereal straws, esparto, corn, bamboo, reed, and a number of lesser-known species such as elephant or Napier grass and Columbum grass. Because of the close relationship of these species, a similarity in their cellular elements might be anticipated. The entire stem of these plants, together with the leaves in the case of grasses and straws, is utilized, with a resultant variety of characteristic cell types evident in the pulp. Some of the cells which distinguished this group from others used in the manufacture of paper include: relatively short fibers from the vascular bundle; cells of the ground tissue which are barrel-shaped to narrow-cylindrical; various vessel segments of the annular, spiral, and pitted types; and epidermal cells, either in masses or as individuals, easily recognized because of their more or less irregular margins, especially when characterized by prominent tooth like projections.

10.1.1 *Sugar cane, saccharum officinarum* (Figs. 3, 4, 5, and 6). The vascular bundle fibers of this species vary in cell wall diameter and are with pointed ends (Figs. 4 and 5). Except for occasional small pits, there are no characteristic wall markings. Furthermore, thin-walled, short, fiber like cells with oblique, blunt, or even forked ends may be present. Cells of the parenchyma type, ranging up to 850 μm long, and 140 μm wide, and individual vessel elements as long as 1350 μm and as wide as 150 μm are distinctive due to their large size (Fig. 4). Narrow, rectangular, epidermal cells with slightly undulating margins are present in whole, unfractionated stem material (Fig. 3) but only sparingly present in depithed material. The guard cells accompanying the stomata are smooth walled.

10.1.2 *Cereal straws: barley, Hordeum sp.* (Fig. 7); oat, *Avena barbata* (Fig. 8); rye, *Secale sp.* (Fig. 9); wheat, *Triticum sp.* (Fig. 10); triticale, *Triticale sp.* (Fig. 11); emmer, *Triticum dicoccum* (Fig. 12). The fibers of these cereal straws are somewhat longer and wider than those obtained from esparto. The fiber lumen may be narrow or broad, and the fiber ends are pointed. Cells of the ground tissue, barrel-shaped and cylindrical (Figs. 8, 11, and 12), varying up to 450 μm in length and 130 μm in diameter are abundant. Vessel elements, also, are extremely variable in size, ranging up to 100 μm by 60 μm , but fall considerably short of the maximum diameter attained in corn and sugar cane. Sparsely pitted epidermal cells are frequent and variable. Their margins are characteristically marked with prominent teeth (Figs. 8, 10, and 12), but often these are reduced to exhibit a slightly undulating surface. Epidermal cells vary from 36 to 445 μm in length and from 10 to 41 μm in width on the surface. The guard cells surrounding the stomata are smooth-walled. Although inconspicuous erect trichomes may be present, their occurrence is far less frequent than in esparto or rice.