THE RELATIONSHIP OF EXTRACTIVE CONTENT TO PARTICLE SIZE DISTRIBUTION IN MILLED YELLOW-POPLAR (*LIRIODENDRON TULIPIFERA* L.) BARK[1]

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ABSTRACT

A method of separating milled yellow-poplar (*Liriodendron tulipifera* L.) bark into five sieve fractions containing representative inner and outer bark, cells, tissues, and fragments was developed. Anatomical composition of the fractions was examined by light microscopy. Each fraction was sequentially extracted with ether, ethanol-benzene, hot water, and cold water to investigate the relationship between the extractive content and the anatomical composition of the fraction. A statistically significant relationship was found between the amount of extractive and the particle size distribution of material in each fraction. Additionally, extractive content was found to be dependent not only on the absolute amount of bark retained but also on the relative amounts of inner and outer bark in each fraction. Total extractive content was found to increase with decreasing particle size. Examination of SEM micrographs following each stage in the extraction process revealed sequential changes to cell structure. The magnitude of the changes appeared to be closely related to the amount of extractive recovered in each fraction. Changes in porosity and void volume, as an indication of the removal of extractives by fraction, were investigated with a mercury porosimeter. Data indicated significant and sequential changes in porosity and void volume following extraction.

Keywords: Tulip-poplar, bark, anatomy, particle size, extractives, SEM, porosimetry.

In 1963 it was pointed out that "relatively little attention has so far been paid to the connection between the anatomy of bark and its chemical composition" (Jensen et al. 1963). Almost two decades have passed and little has happened to alter that observation. Continued emphasis is placed on the full utilization of bark as a chemical raw material, yet little information exists on the relationship between anatomy and chemical composition. The overall anatomy of bark is discussed by Chang (1954), Esau (1965), and Martin and Crist (1970); and the specific nature of yellow-poplar bark is thoroughly discussed by Cheadle and Esau (1964). The general chemical composition of bark may be found in Kurth (1949) and

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Jensen et al. (1963). Approximately 14 million tons of bark, on an oven-dry basis, are generated annually in the United States as residues from primary wood processing (Currier and Laver 1973). In hardwoods some 9 to 15% by volume of the tree is bark, and it has been estimated that, by weight, 20 to 30% of bark consists of chemical extractives. Among the many uses suggested for bark is its potential as a source of industrial chemicals (Farber 1959; Goldstein 1975). Goldstein (1975) suggested that a major portion of this country's synthetic polymer feedstock needs might be supplied from the cellulose and lignin components found in wood and bark. The full development of the chemical potential of the tree may best be realized by considering the bark as a separate entity.

A compendium of analytical information on the extractive contents of bark by species is needed to realistically gauge its useful potential. Bibliographic information on the content of many barks exists, and recent works present detailed extractive information on many eastern hardwood species (Harkin and Rowe 1971; Ross 1966; Rowe and Conner 1979). To date, however, there is neither a widely accepted common method for analyzing nor a unified scheme for separating bark into its anatomical or chemical components prior to analysis. Types and amounts of extractives recoverable from bark depend on the chemical composition of the individual cells and tissues that make up the material and thus are also dependent on means of preparation and separation of the raw bark particles.

Many methods have been devised to isolate the various types of cells and tissues found in bark. None have been standardized, but the methods commonly used are divided into three groups: mechanical, electrical, and chemical separation. The first step in any process is to remove the bark from the tree by any one of several mechanical debarking procedures. Each debarking process produces a bark of a singular fiber or particle nature. Mechanical methods of separation of the raw bark are usually of the type described by Kurth and Smith (1954). This involves fragmenting the bark followed by mechanical separation of the fragments by sieving. Electrical separation of previously milled bark particles is discussed by Short et al. (1973). Here, milled particles are placed in an electric field wherein fibers are separated from cork cells and fines. The procedure is dependent on the geometry of the beginning material, and separates only fiber bundles that are very long relative to the granular shape of the cork particles. Chemical separations, principally of the type that dissolve one cell type or tissue leaving another, may also be carried out (Gregory et al. 1962). This procedure, however, might significantly alter the chemical nature of the residual material.

Ideally, a process should be developed that efficiently and reliably separates bark into its component cells and tissues without chemically altering the beginning material. A mechanical separation method whereby bark is reduced to a series of particle size classes reasonably representing a separation among naturally occurring cell types or tissues would be useful. This approach would be especially helpful if isolations of extractives known to be associated with a specific cell type or tissue were desired.

This paper describes a means of separating milled bark wherein the relationship between sieve fraction particle size and predominant cell or tissue type combinations or fragments is established. Further, the relationship between extractive content, particle size, and inner or outer bark content is investigated. Finally, the effect of extraction on cell anatomy, by particle size, of inner and outer bark

utilizing both scanning electron microscopy (SEM) and mercury porosimetry studies is presented.

MATERIALS AND METHODS

Yellow-poplar (*Liriodendron tulipifera* L.) bark specimens of dimensions 2.5 cm (longitudinal) by 0.6 cm (tangential) by 2.0 cm (radial) were prepared from samples that were held at 0 C following collection, were thawed, and then finally air-dried. Bark specimens, hereafter termed whole bark, were randomly selected and ground to pass a 2.0-mm retaining screen in a No. 4 Wiley mill. Other specimens were divided into inner and outer bark and were similarly ground.

Sieving

Wiley-milled samples were separated into five particle fractions using U.S. Standard sieves 203 mm in diameter (ASTM E 11 specification). The sieve fraction series consisted of mesh sizes 18 (particles greater than 1.03-mm diameter), 35 (0.52 to 1.03 mm), 60 (0.29 to 0.52 mm), and 120 (0.14 to 0.29 mm) (Tyler 1973). Particles smaller than 120 mesh were retained in a bottom pan and were termed "fines." The sieving procedure was begun by placing 50 g of either whole, inner, or outer air-dried bark in the topmost 18 mesh sieve. The sieves were vibrated at 270 oscillations per minute in an Autovert shaker for a total of 15 min. To circumvent an undesired alignment of particles and fibers that might prolong or make the separation process less than complete, the vibration was stopped every 3 min and the sieves were rotated 90 degrees in a clockwise fashion. At the end of the shaking period, the oven-dry weight of the material on each sieve fraction was expressed as a percent relative to the oven-dry weight of the original Wiley-milled whole bark. Representative samples from each sieve fraction were examined with a light microscope to ascertain their anatomical composition. Samples were then conditioned to an equilibrium moisture content of 4% in preparation for the next stages of the analysis.

Extraction

Samples of whole, inner, and outer bark from each mesh size were sequentially extracted following TAPPI procedures, modified as described below to accommodate small sample amounts, using ethyl ether (T5 m-59), ethanol-benzene (T6 os-59), and cold, then hot, water (T1 os-59) (TAPPI 1959). Soxhlet microextractors with a 0.5-g sample and 20-ml of solvent were used for the organic solvent extraction, while 0.5 g of sample and 75 ml and 25 ml of cold and hot water, respectively, were used for water extractions. The organic extractives were reduced to dryness in a rotary evaporator *in vacuo,* while the water extractives were dried under a warm air stream. The extractive content for a mesh size was expressed as a percentage of the oven-dry weight of the unextracted bark. In addition, the amount of extractive based on the relative contribution of various tissues, cells and fragments, by sieve fraction, was expressed as extractive yield and was calculated as follows:

$$\text{Extractive yield for each solvent (\%)} = \frac{(A)(B)}{\Sigma_n[(C)(D)]} \cdot 100$$

where: A = Amount of bark retained on a given mesh size
B = Extractive content of a given mesh size
C = Amount of bark retained for a mesh size
D = Extractive content for a mesh size
n = Summed over all mesh sizes

Extractive yield varied with the solvent system and the types and amounts of particles present in each fraction. All extractive treatments were randomly allocated with respect to mesh size, and a factorial experimental design with eight replications was used.

Scanning electron microscopy

Extracted as well as unextracted whole, inner, and outer bark samples were examined using an International Scientific Instrument Co. Model 60 scanning electron microscope (SEM). Samples were coated with gold and were examined under an accelerating voltage of 15 KV. Representative SEM micrographs were taken following each step in the extraction process and were examined to investigate possible changes in anatomical structure due to extractive treatment.

Porosimetry

Samples that had been air-dried following organic solvent extraction and vacuum-dried samples that had been extracted with cold and hot water were investigated with a mercury porosimeter to detect porosity and void volume changes. The samples were weighed, nominal weight of 0.01 to 0.02 g, placed in a 2-ml graduated penetrometer, transferred to the vacuum chamber of the porosimeter, and subjected to a vacuum of 50 μm. The vacuum was slowly released to a point where mercury was just drawn into the penetrometer. At this point the pressure was increased in increments first to fill the voids, at about 300 psi (2.06×10^4 kPa) and finally to fill the smallest pores, at about 15,000 psi (1.03×10^5 kPa). At that point the sample was assumed to be completely filled with mercury. A factorial design was used to investigate the effect of sieve fraction and solvent type, using eight replications and a fixed effects model.

RESULTS AND DISCUSSION

Sieve analysis

An analysis of variance of the particle size distribution of milled whole bark as well as the inner bark fraction by mesh size was significantly different at the 0.01 level. Approximately 62%, based on oven-dry weight, of the Wiley-milled bark was found to consist of inner bark, while 38% was outer bark (Table 1). These values are similar to the range of weight values calculated from volume data presented by Koch (1971) for yellow-poplar bark. An increase in the amount of inner bark was found to occur with decreasing particle size. Differences in the percentage of particles by mesh size was found to be significant among the 18, 35, 60, and a sieve category in which the 120 and fine fractions were combined. This general finding is consistent with that for Douglas fir bark (Kurth and Smith 1954). The process of rotating the sieve series through 90 degrees every 3 min during the separation procedure insured consistent particle distribution by sieve fraction. It is believed that rotation forestalls possible particle alignment parallel

TABLE 1. *Particle distribution of Wiley-milled whole, inner, and outer bark fractions by mesh size.*[1]

Mesh size	Particle source				
	Whole bark	Inner bark		Outer bark	
		%			
18	1.01	0.19[2]	0.32[3]	0.82[2]	2.14[4]
35	26.41	8.23	13.37	18.18	47.31
60	34.53	23.41	37.97	11.12	28.99
120	22.66	17.46	28.33	5.20	13.56
Fines	15.39	12.32	20.01	3.07	8.00
Total	100.00	61.61	100.00	38.39	100.00

[1] All computations based on oven-dry weight.
[2] Percentage on basis of total (whole) bark.
[3] Percentage on basis of total inner bark.
[4] Percentage on basis of total outer bark.

to the direction of shaking. Resumption of shaking at right angles to the previously aligned bark material appears to allow more separation of the smaller particles that might otherwise be restricted from moving downward to the sieve openings in the 15-min period.

Particle distribution of inner and outer bark is shown in Table 1 as a percentage of total bark, as well as percentage of either total inner or outer bark by sieve fraction. This method of quantifying particle distribution is useful, because it not only indirectly indicates the type of anatomical distribution by sieve fraction but also provides the relative quantities of inner and outer bark based on the total amounts of these categories present. These values have importance because they may be used to locate that particle fraction from which a maximum amount of specific tissue may be recovered. In this study the following types of cells, by mesh size, predominated: cork cells, 18, 35 mesh; fibers, 60 mesh; fibers and parenchyma, 120 mesh; parenchyma fragments in the fine mesh fraction. Recovery of a specific tissue might thus lead to the more rapid isolation of a desired chemical fraction. For instance, it has been postulated that thin-walled phloem parenchyma serve in the storage of materials such as tannins, resins, and oxalic acid (Stewart 1960). Analysis by chemical extraction for these materials might well begin with utilization of bark particles retained on both the 60 and 120 sieve fractions, as those fractions constitute 66% of the total inner bark cell types and tissues present. Further separation of contaminating outer bark elements, e.g., cork cells, might then be carried out by a floatation method similar to that of Van Beckum and Miller (1960). Light microscopic examination of each sieve fraction verified the presence of the predominant cell types, tissues, and fragments expected to be associated with the inner or outer bark anatomical constituents.

Extraction

Total extractive content increased with decreasing particle size for all solvents (Fig. 1). The extractive yield (Fig. 2) for each sieve fraction depends not only on the absolute extractive content of all particles in that fraction but also more importantly on the relative amounts of inner and outer bark (Table 1) with their associated cell and tissue types. The extractive yield for a sieve fraction and solvent is high if both the extractive content of the cells or tissues from that

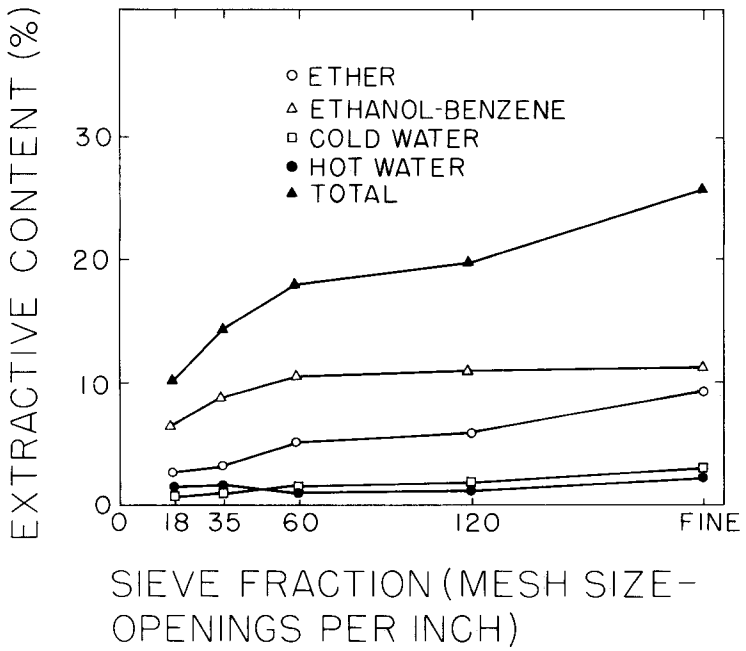

FIG. 1. Extractive content by solvent type and sieve fraction.

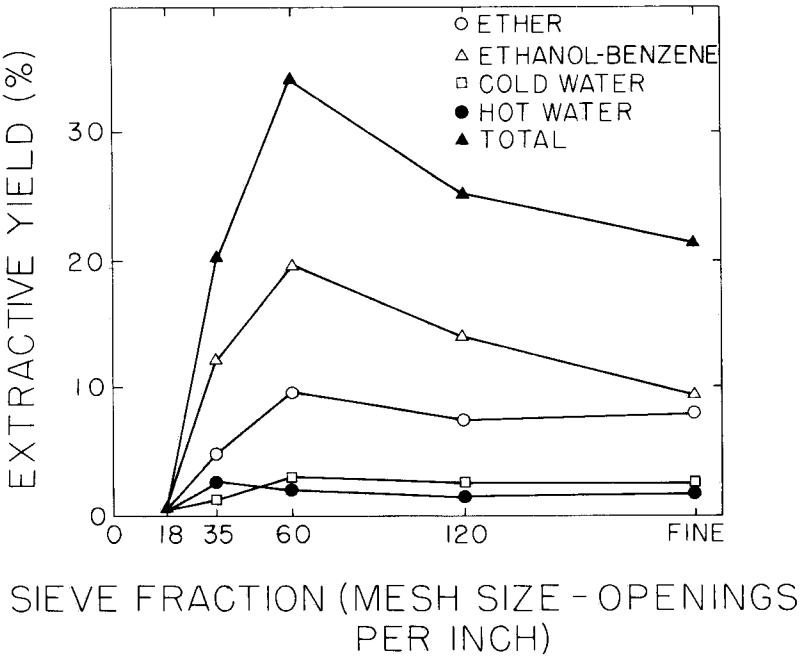

FIG. 2. Extractive yield by solvent type and sieve fraction.

fraction is high and if the sieving procedure isolates relatively large amounts of inner bark particles. Thus, although the total extractive content of the 60 mesh fraction was equal to or slightly lower than that of the 120 mesh or fine fractions (Fig. 1), the combined amount and type of the particles isolated on the 60 mesh sieve was sufficient to produce a higher extractive yield (Fig. 2). An analysis of variance indicated that both extractive content and yield differed significantly with respect to mesh size and solvent type.

The observed general increase in extractive content with decreasing particle size may be attributed to the increased particle surface area to volume ratio of the finer particle fractions or may occur because of the presence of many more extractive rich cells or fragments. Solvolysis by both ether and ethanol-benzene was particularly high in the 60 mesh fraction. This fraction contained the greatest amount of inner bark, a tissue particularly high in ray tissue, and thus in extractives. Light microscopic examination of the 120 and fine sieve fractions revealed that these fractions contained large amounts of fragmented inner bark tissue, which gave the highest extractive content values. The greatest outer bark content was found in the 35 mesh fraction. Hot water treatment produced a higher extractive content and yield in the 18 and 35 mesh fractions than did the cold water treatment. As particle size decreased, however, and tissue and cell types changed and as the amount of fragmented material increased, cold water was a more effective solvent. This may be due to an increase in the amount of inner bark which contained extractives soluble in cold water, or perhaps to the increase in the number of cell particles or fragments with labile sites now exposed to cold water. A reversal of the water extraction sequence resulting in first hot, then cold water treatments would in all likelihood leave few cold water extractives.

Scanning electron microscopy

Extractive compounds particularly starch, callose, ergastic substances, crystalliferous material, and phenolic substances are known to be deposited in the bark (Esau 1965; Chang 1954; Kurth 1949). Removal of these compounds may be expected to have a marked effect on overall cell topography and perhaps cell-wall structure. Scanning electron micrographs of inner and outer bark prior to and following extraction (Figs. 3–6) indicate these removal effects. The most apparent changes in cell structure occurred in the ray parenchyma tissue of the inner bark (Figs. 3, 4). The results following the final step in the extraction sequence revealed well-delineated individual ray parenchyma cells previously occluded by extractives. This observation is consistent with the finding that the 60 mesh fraction, a fraction composed mainly of inner bark, had a high extractive content. Additionally, these changes would be expected from the 120 and fine sieve fractions. However, this was difficult to confirm visually because of the fineness of the highly fractured material.

Noteworthy changes also occurred in the cell-wall morphology of outer bark particles (Figs. 5, 6). Alterations in cell anatomy occurred because of the loss of material deposited within the lumens of cells, as well as the absence of encrustations on the cell walls following extraction. The thickness of the cell walls were decreased on extraction and this reduction in cell-wall thickness is readily seen in comparing the extracted to the unextracted tissue. Materials deposited in the lumens and encrustations present in the unextracted cells have been removed by

FIG. 3. A tangential view showing a portion of an unextracted secondary phloem ray completely occluded by extractives. Note that individual cell walls and lumens are indistinct. Inner bark tissue, 60 mesh sieve fraction, the left-hand marker is 10 μm.

the extraction process. In many instances cell walls seem to have been cleaved, fractured, or totally dissolved, although this may be an artifact caused by mechanical milling, sieving, or vacuum drying in preparation for SEM observation. Many of the SEM micrographs showed that pores and pore areas not visible prior to extraction become visible following hot water extraction.

FIG. 4. A tangential view showing a portion of a completely extracted secondary phloem ray. Note now that individual cell walls and lumens are distinct. Inner bark tissue, 60 mesh sieve fraction, the left-hand marker is 10 μm.

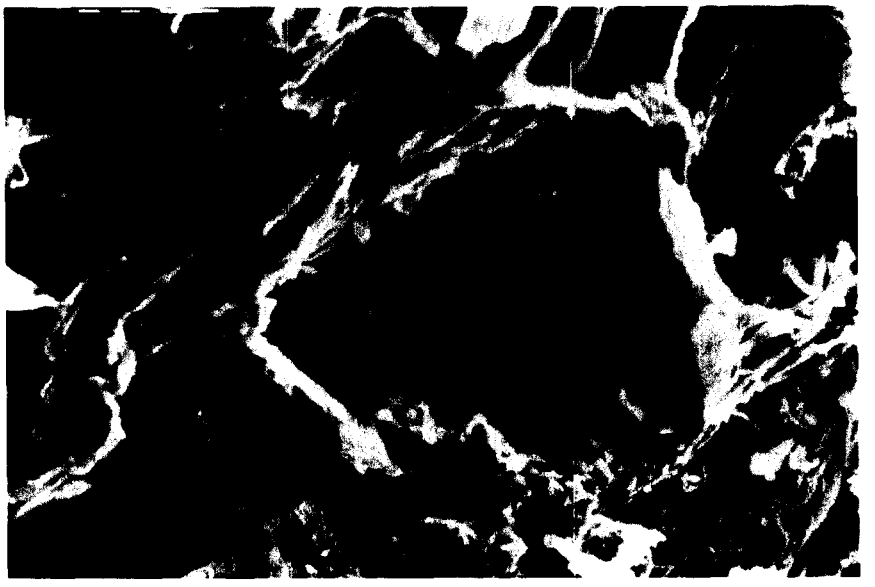

FIG. 5. Unextracted cork cells. Note cell walls are relatively thick and rough areas of encrustations in the lumens are apparent. Fractures in walls may be due to milling or drying procedures. Outer bark tissue, 60 mesh fraction, the left-hand marker is 10 μm.

Porosimetry

Porosimetry yields data on porosity, i.e., the amount of void volume in a porous sample that may be expressed as a percentage of the total volume. Mercury penetration detects not only pores and voids within particles of a ground sample,

FIG. 6. Completely extracted cork cells. Note that cell walls are now considerably thinner and are sculptured where extractives have been removed. Outer bark tissue, 60 mesh fraction, the left-hand marker is 10 μm.

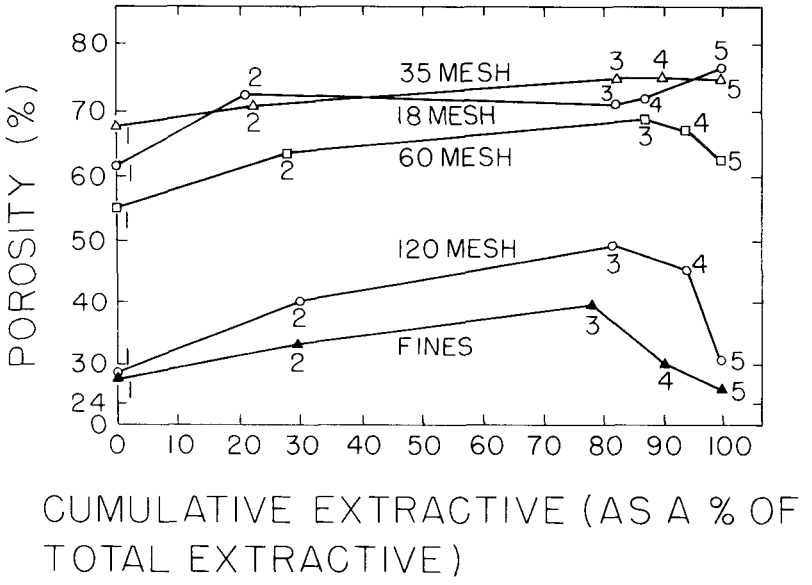

FIG. 7. Percent porosity on basis of total extractive by mesh size. 1) unextracted, 2) ether, 3) ethanol-benzene, 4) cold water, 5) hot water.

but also the void volume between fragments and particles. The space between particles is not considered to be part of the pore volume. Misinterpretation of data representing mercury penetration of these interstices would introduce large errors into the study. Changes in porosity as a function of sequential extraction of the bark by sieve fraction are indicated in Fig. 7. The cumulative extractive percent value represents the amount of extract removed from the bark by each solvent in the series up to and including the given solvent and is expressed as a percentage of the total amount of extract present. As mesh size decreased, a greater percentage of the material retained on each sieve fraction consisted of fractured cells and tissues. Thus, as the amount of intact cells and tissues decreased, so did the porosity.

The porosity within each sieve fraction generally increased with the removal of the ether and ethanol-benzene soluble compounds, reflecting solvolysis of extractive materials deposited in cells and on cell walls. Increased porosity resulted from increased lumen volume, the dissolution of encrustations that occlude pits and pores, and voids created in the cell wall by the removal of bulking extractives. After an initial increase in porosity following ether extraction in the 18 and 35 mesh outer bark fractions, little change was observed. These fractions are composed primarily of cork cells and tissues and the major portion of the waxes, suberin, and hydroxy acids was not removed by the solvent series. The rapid decrease in porosity of the 60, 120 and fine series following water extraction is believed to be an artifact of preparation of the samples for the porosimeter. Examination of the SEM evidence (Figs. 5, 6) indicates that many extracted cells are cracked and broken. This occurrence is most likely due to a combination of milling and the removal of extractives as well as the rather severe drying and vacuum conditions necessary to prepare samples for both the porosimeter and

SEM. Perhaps the thickened and incompletely extracted cork cell walls of the tissue comprising the 18 and 35 sieve fractions were better able to resist the stresses of mechanical abrasion and vacuum drying than were the thin-walled, inner-bark cells found in the other three fractions.

SUMMARY

A reliable method of processing Wiley-milled bark by sieving produced sieve fractions containing representative inner and outer bark cells, tissues, and particles. A statistically valid relationship was found to exist between the amount of extractives recovered from a standard extraction sequence and the particle size distribution in the various sieve fractions. The extractive content of the bark was found to increase with decreasing particle size. In each sieve fraction, the extractive content depended not only on the absolute amount of material retained on the sieve but also on the relative amounts of inner or outer bark in that particular sieve fraction. A comparison of unextracted to extracted samples by examination of SEM micrographs at each stage throughout the extraction sequence showed significant changes in the amount of materials deposited in the cells and on the cell walls. Extracted ray parenchyma tissue and cells from inner bark showed the most striking changes, while cork tissue from outer bark showed noticeable changes in overall anatomy and cell-wall thickness. Porosimetry data indicated significant and sequential changes in porosity and void volume space following extraction. The porosity of each sieve fraction generally increased with removal of the ether and ethanol-benzene extractive. The decrease in porosity observed following water extraction is believed to be due to an artifact of sample preparation.

The sieving procedure outlined in this paper appears to provide a rapid and reliable means of separating representative samples of the inner and outer bark of yellow-poplar. Sequential extraction followed by SEM observation and examination by porosimetry of the extracted bark established the relationship between extractive content and particle size caused by different proportions of cells and tissue types in the various sieve fractions.

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