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Variation in Surface Chemical Constituents of Cotton (*Gossypium hirsutum*) Fiber as a Function of Maturity

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Modern cotton yarn production technology has made it imperative that new predictors of yarn spinning efficiency be determined. Surface frictional forces play a large role in spinning efficiency, yet little is known about the chemical constituents comprising the cotton fiber surface or their respective roles in inter-fiber frictional behavior. Major cotton fiber surface chemical components including pectin, wax, soluble salts, and sugars were quantified, and their respective relationships to cotton fiber maturity, as measured by micronaire, determined for 87 cotton samples exhibiting large variations in age, micronaire, genetics, and growing region. In the case of pectin and wax, inverse relationships with micronaire were found, whereas salts and sugars exhibit linear relationships with micronaire. Using these mathematical relationships, it will be possible to develop predictive models of whether spinning performance of different cottons is affected by deviations of the chemical constituents from the determined relationships.

KEYWORDS: Cotton; *Gossypium hirsutum*; fiber; friction; maturity; micronaire; pectin,; wax; sugar

INTRODUCTION

Over the past several years, improved cotton yarn production technology has resulted in large increases in production rates. As a result, it has become increasingly apparent that the standard high volume instrumentation (HVI) measurements of fiber properties such as length, strength, and uniformity are no longer entirely satisfactory as predictors of yarn spinning efficiency (*1*). It is generally accepted that spinning performance is affected by the surface characteristics of cotton fibers (*2*), which can in turn affect inter-fiber frictional properties. Several factors contribute to the surface frictional forces between fibers, including fiber morphology (convolutions), geometric features (length, fineness), and static electrical forces. In addition, the surface of the fiber is comprised of a primary cell wall composed chiefly of pectin and hemicellulose, a cuticle composed of waxes, and surface residues including salts and sugars. Because these outer surface components are in direct contact with neighboring fibers, the physical dimension and chemical compositions of these surface components may potentially have a much larger impact upon spinning performance than is reflected by their low overall abundance relative to cellulose. It has been demonstrated (*2*) that increased wax content is correlated with decreased fiber−fiber and fiber−metal friction as determined by the modified rotor ring energy. Similarly, an increased quantity of calcium present in the fiber has also been correlated with decreased fiber−fiber and fiber−metal friction (*3*). Calcium in cotton fiber is associated with the primary cell wall, where it serves to cross-link pectin polymers. Increased pectin content is thus indirectly correlated with decreasing fiber friction.

To determine the factors involved in spinning performance, it is necessary to further elucidate the surface properties of cotton. Surface frictional properties are likely to be directly correlated with quantities of surface chemical constituents. In addition, qualitative differences in these substances might be expected to vary as a function of genetics and environmetal factors. Recently (*4*), wax content was determined for a diverse group of cottons and compared with HVI properties, and the relationship with micronaire was the most significant correlation found. In the micronaire instrument, a weighed quantity of 3.24 g of cotton sample is compressed into a cylindrical container of fixed dimensions. Compressed air is forced through the sample at a definite pressure and the rate of flow of air is measured. The resistance offered to the flow of air through a plug of fibers is dependent upon the specific surface area of the fibers. The specific surface area determines the flow of air through a cotton plug is dependent upon the linear density of the fibers in the sample. Micronaire is thus a measure of fiber linear density and is related to both fiber maturity and fiber fineness. As a measure of fiber maturity, micronaire effectively measures the degree to which the secondary cell wall has been established. The secondary cell wall is comprised nearly exclusively of cellulose and accounts for approximately 95% (w/w) of the raw cotton fiber. Because growth of the secondary wall is initiated following the establishment of the primary cell wall and cuticle, the proportions of chemical components comprising the primary cell wall and the cuticle might be expected to decrease as the fiber matures and the micronaire value increases. This has been observed (*4*) in the case of wax,

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and it is thus reasonable to assume that surface chemical components associated with the primary cell wall will also have some dependence upon micronaire. Quantities of a surface component falling above or below the average value expected at a given micronaire value may indicate a change in layer thickness (or density) potentially affecting frictional properties. The purpose of this work is to examine the quantitative dependence of pectin, waxes, metals, and soluble sugars and salts upon micronaire. Knowing the variation expected as a function of micronaire will subsequently help to elucidate potentially important differences in surface chemistry that are controlled by factors other than micronaire such as genetic variation, environmental influences, etc.

MATERIALS AND METHODS

For this study, a total of 87 cotton samples exhibiting a wide range of genetic diversity, micronaire, growing regions, and storage times were chosen.

Micronaire was measured by high volume instrumentation (HVI) according to standard test methods (*5*).

Cotton fiber pectin content was determined by enzymatic degradation and subsequent analysis of galacturonic acid, the primary byproduct of enzymatic degradation. Enzyme treatments of cotton samples consisted of adding 10 μL of pectinase (Sigma Chemical Co.) to a 0.10-g cotton sample in 10 mL of a pH = 4.0 buffer solution. To this suspension was added 0.01 g EDTA, with subsequent heating at 50 °C for 18 h. For galacturonic acid analysis, high performance anion exchange chromatography (HPAEC) was performed on the resultant suspensions with a Dionex DX-500 using pulsed amperometric detection and two Dionex Carbopac PA-1 (4 × 250 mm) columns connected in series. Elution was carried out at 0.75 mL/min using 200 mM NaOH as the mobile phase and a sigmoidal gradient of 0−500 mM NaOAc. Galacturonic acid content is reported as the fraction (w/w) present on the fiber.

Wax contents, reported as the fraction (w/w) present on the fiber, were determined by Soxhlet extraction. Extractions were performed using trichloroethylene as solvent on 2.5 g samples of cotton. The resultant solutions were evaporated to dryness at 105 °C, leaving a wax residue, which was subsequently weighed.

Cotton samples were extracted using 20 mL deionized water per gram of cotton. Each sample was agitated with a glass rod to promote wetting of the cotton surface. The resultant wetted sample was allowed to sit for 15 min before being wrung out. The resulting extract was then subjected to conductivity measurements, pH measurements and glucose measurements. Conductivity measurements, reported in microsiemens per centimeter (:S cm^{-1}), were performed on a Myron L Company Model EP conductivity meter. Glucose measurements, reported as the fraction (w/w) present on the fiber, were performed using a Yellow Springs Instrument Co. Model 2700 Bioanalyzer equipped with glucose oxidase membrane. Measurements for pH were performed on an Orion model 310 pH meter.

Linear and nonlinear analysis of data was performed using SigmaPlot 5.0

RESULTS AND DISCUSSION

Enzymatic degradation of cotton fiber pectin using pectinase results in the observation of galacturonic acid as the primary component using HPAEC as the method of analysis. Because 100 mM NaOH is used as an eluent, any esterified galacturonic acid present following pectinase treatment is converted to acidic galacturonic acid. Previous research (*6*), however, indicates that little esterified galacturonic acid is present in cotton fiber pectin. It is therefore concluded that cotton fiber pectin is primarily composed of polygalacturonic acid with a significant but undetermined number of cross linkages due to calcium. Pectin is a major component of the primary cell wall in the cotton fiber, comprising approximately 25% of the cross-sectional area,

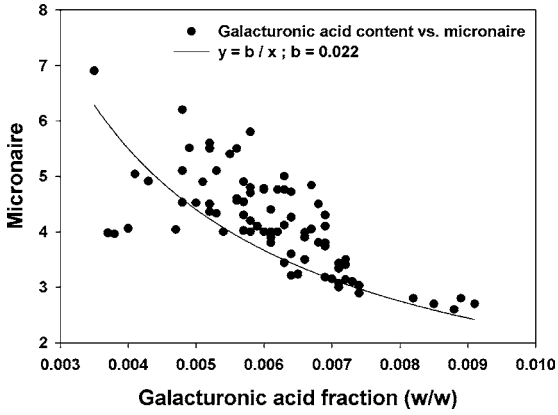

Figure 1. Comparison of galacturonic acid fraction with micronaire for 87 cotton samples.

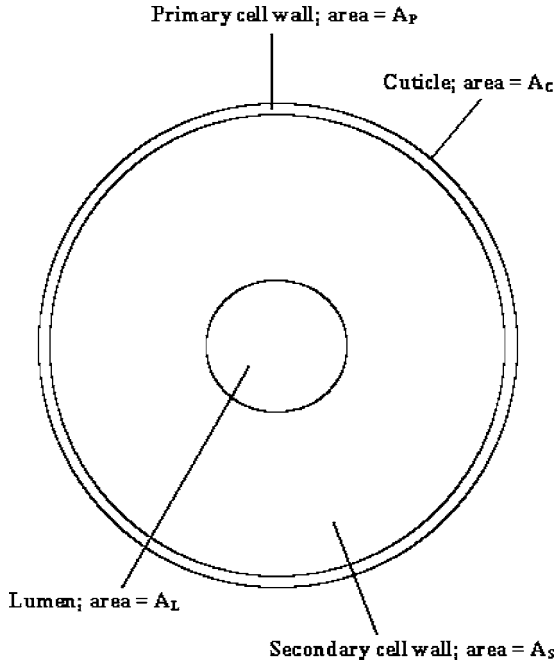

Figure 2. Diagramatic representation of a cotton fiber cross-section.

(*7*) and because it is on the outer surface of the fiber cell wall (*6*), it can potentially play a significant role in surface frictional properties. A comparison of fiber galacturonic acid content with fiber micronaire is shown in **Figure 1**. A linear fit of the data gives a good correlation of increasing galacturonic acid with decreasing micronaire, with $R^2 = 0.51$. A linear fit, however, implies that the pectin component, seen in **Figure 2** with cross-sectional area A_P, decreases as the total area excluding the lumen, $A_T = A_P + A_S + A_C$, increases. A_T describes the cross-sectional area of the fiber due to all components including pectin, wax (cuticle), and cellulose and is proportional to the measured micronaire value. This linear relation is described by

$$\text{Micronaire} = b - mx$$

where x is the fraction (w/w) of galacturonic acid and is proportional to A_P/A_T, b is the y axis intercept at $x = 0$, and m is the slope. Two implications of this linear relation are the constraints (1) that as A_T approaches b, A_P approaches 0, and (2) as A_T approaches 0, A_P/A_T approaches 0.013 ($552x = b$). Neither of these constraints is valid from what is known about fiber growth. Previous research (*7*) has shown that the primary wall is formed prior to the deposition of the secondary wall

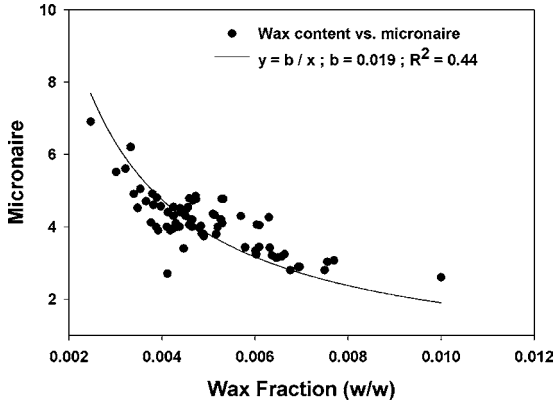

Figure 3. Comparison of wax fraction with micronaire for 87 cotton samples.

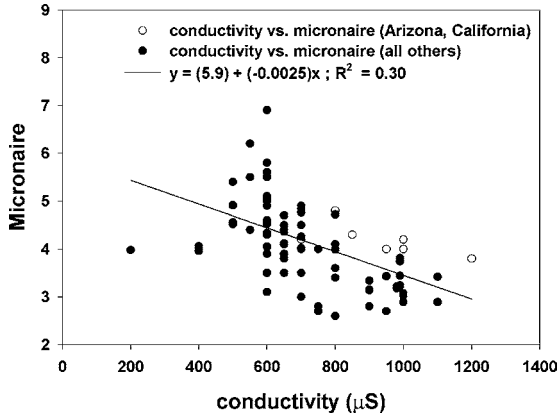

Figure 4. Comparison of conductivity with micronaire for 87 cotton samples.

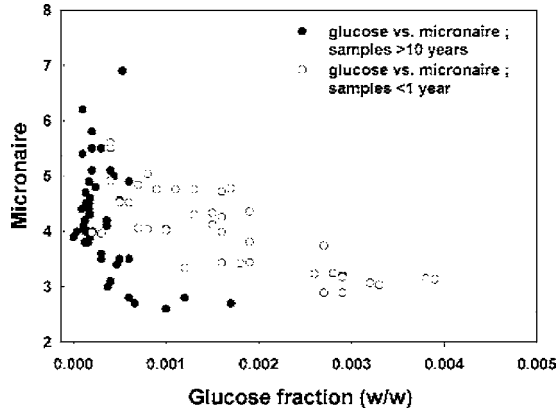

Figure 5. Comparison of glucose fraction with micronaire for 87 cotton samples.

(with some overlap) and that once formed does not degrade, but remains constant. Even though the fiber diameter may change (*8*), the cross-sectional area of the primary wall, A_P, probably does not change. Assuming that A_P is constant, then the relation $A_P/A_T = x$ becomes (b/x = Micronaire). This relation more properly models the situation where the cross sectional area of the pectin component is constant while the secondary wall is formed. The two constraints (a) x approaches 1 as A_T approaches A_P, and (b) A_T approaches infinity as x approaches 0, are also more in keeping with observation. **Figure 1** shows the curve (Micronaire= b/x) with $b = 0.022$. This curve approximates the boundary describing the minimum amount of galacturonic acid as a function of micronaire. Those points falling to the left of the curve have lower values due to possible microbial degradation, or cavitoma (*9*), which is further evidenced (vide infra) by low glucose levels and high pH levels. The cross-sectional area of the pectin layer at the onset of secondary wall growth may vary depending upon a number of potential factors including genetics, environment, and maturity. The minimum value of galacturonic acid at a given value of micronaire is represented by $b = 0.022$. This boundary represents the minimum of pectin growth at the initial onset of cellulose deposition.

Figure 3 shows a comparison of wax content to micronaire. As with the pectin layer, it is assumed that the wax layer, or cuticle, is formed before the onset of secondary wall formation, and that the fraction of wax on cotton fiber follows (Micronaire $= b/x$), where $x = A_C/A_T$. This curve, with $b = 0.019$, is displayed in **Figure 3**.

Extraction of raw cotton lint using deionized water results in a solution exhibiting a marked increase in conductivity compared to deionized water. This suggests that conductive salts are present on the surface of cotton, whether they reside in the inner lumen or on the outer surface of the fiber. This supports earlier work indicating that several cationic species are present in the ash of cotton lint (*10*). Those cations present include potassium, magnesium, calcium, and sodium. Of these, potassium is by far the most prevalent, comprising approximately 80% (w/w) of the total cations present. The anion profile of the cotton water extract is not as well established, but malate has been identified (*11*) as an important metabolic species present in the vacuole during growth, and this species is presumably present as a residue, along with potassium and other cations and anions, after drying.

To determine the relationship between potassium concentration and conductivity, solutions of potassium citrate, potassium malate and potassium chloride were measured for conductivity at a variety of concentrations. Results indicate that conductivity can be related to potassium salt concentration by the equation

$$\kappa = \kappa_0 + ac + bc^2;\ \kappa_0 = 0,\ a = 0.2,\ b = .000003$$

where c is the concentration of potassium salt, κ is the conductivity, κ_0 is the conductivity at $c = 0$, and a and b vary depending upon anion type. At the concentrations observed in the cotton extracts used in this study (4000 ppm and less), the relationship is approximately linear, with

$$\kappa = \kappa_0 + \mathrm{ax};\ \kappa_0 = 0,\ a = 0.2$$

and variation due to different anions is relatively minor. As a consequence of this approximate linearity, conductivity measurements were utilized in this study as an indirect measure of potassium concentration due to the simplicity of the method. One of the functions of potassium malate and other salts present in the vacuole during fiber growth is to provide turgor pressure to prevent cell collapse. Once fiber growth is terminated, the vacuole dries out, leaving a residue of salt, sugar, etc. For lower micronaire (less mature) cottons, the lumen,or vacuole, occupies a larger cross-sectional area on a basis relative to A_T and holds a larger quantity of dissolved components than higher micronaire cottons. As the secondary cell wall grows, the lumen shrinks. Assuming a constant concentration of potassium malate in the lumen, then the total quantity of potassium malate will decrease as the fiber matures. There is thus a theoretical linear dependence of potassium, and therefore conductivity, with micronaire, based on the formula

$$\text{Micronaire} = b - mx$$

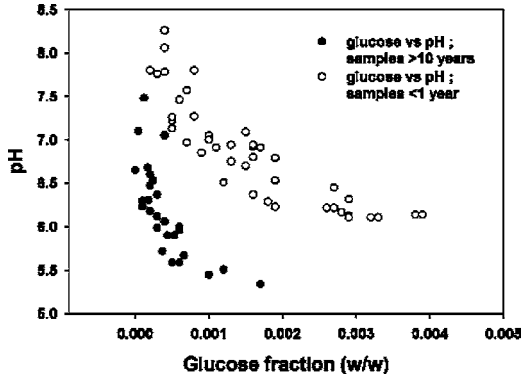

Figure 6. Comparison of glucose fraction with pH for 87 cotton samples.

where b is a constant and describes the total area within the fiber perimeter ($b \propto A_P + A_S + A_C + A_L$), x is the conductivity proportional to A_L, and m is the slope. A comparison of conductivity with micronaire is shown in **Figure 4**, where $b = 5.9$, $m = -0.0025$ ($R^2 = 0.30$). Experimental determination of potassium content as measured by conductivity shows it to exhibit a high variability even at fixed values of micronaire. Some of this variability appears to be related to region of growth. Cotton grown in regions of low rainfall and no overhead irrigation (California, Arizona) have a higher proportion of potassium, on average, due to the fact that the potassium salts are not solubilized and washed off of the open boll. Though the correlation of conductivity with micronaire is low due to this weathering, there does appear to be a real dependence of potassium on micronaire.

Comparison of glucose with micronaire is shown in **Figure 5**. The data in this case is separated into two groups to illustrate the effect of fiber age upon glucose content. Comparison of the two groups indicates a substantial (nearly complete) reduction of residual glucose in the aged fiber, possibly as a result of microbial degradation. Results on one subgroup of aged cotton samples in which reducing sugars were measured in their year of harvest, 1991, confirm that glucose has indeed decreased from an average of 0.0011 in 1991 to 0.00012 in 2002, nearly an order of magnitude decrease. This decrease in glucose as a function of age is associated with a concomitant decrease in pH, possibly as a result of the production of acidic metabolic byproducts due to anaerobic microbial degradation of glucose. This is a phenomenon distinct from cavitoma, where the pH is observed to increase. A comparison of pH with glucose is shown in **Figure 6**. The "youngest" group has a roughly linear relation of glucose with micronaire, as seen in **Figure 5**. This is expected, because glucose will follow A_L, as does the soluble salt component. It seems doubtful that this relation holds as micronaire approaches zero, however, because as the fiber is still growing, glucose concentration is more "dynamic" (*7*).

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