**ABSTRACT**

YORK, KAREN MARIE. Isolation, Characterization, and Application of Tobacco Based Colorants to Cotton. (Under the direction of Dr. Harold Freeman and Dr. Melissa Pasquinelli).

The coloration of textiles with natural dyes, especially those from plants, has persisted over the years, despite the economic and application advantages of synthetic dyes. Factors associated with continuing interest in natural dyes include their biodegradability, a perceived environmental advantage, and exposure to potentially genotoxic precursors that characterize certain azo dyes. On the other hand, the use of natural dyes in textile coloration has required solving challenges associated with their isolation from plant materials, their application to substrates for which they have no inherent substantivity, and their modest fastness properties under end-use conditions.

The present investigation emerged from a growing interest in natural dyes and an interest in identifying alternatives to the use of tobacco for smoking. The presence of color-bearing flavonoids compounds in dried tobacco and the availability of large quantities of tobacco dust waste material from cigarette manufacturing made the examination of textile coloration using tobacco dust extract a logical undertaking. With this in mind, methods for the isolation of colorants from commercial tobacco dust and their use in the coloration of cotton fabric were developed. The light and wash fastness properties of the resultant fabric were determined.

Results from extraction studies indicated that hot water was a suitable solvent for tobacco dust extractions and that 30% by weight extractable material could be obtained using an Accelerated Solvent Extractor. Column chromatography on the extract, followed by LC-MS analysis, established the presence of rutin along with two other unidentified colored
components. Dyeing studies using the water soluble tobacco dust extract showed that coloration of cotton could be achieved using a combination of aluminum sulfate and tannic acid as mordants prior to fabric treatment with the extract. In addition, the dyed fabric obtained had very good washfastness and acceptable lightfastness, making coloration using natural colorants from tobacco dust waste extract a potentially viable use of dried tobacco.

In a final set of experiments, it was shown that aqueous tobacco dust extracts contained 3.1% nicotine while the subsequent dyed fabric contained 0.03% (owf) nicotine before washing and 0.0006% after washing.
Isolation, Characterization, and Application of Tobacco Based Colorants to Cotton

by
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DEDICATION

Dedicated to Ben York.
BIOGRAPHY

Karen York grew up in Battle Creek, MI. She attended Michigan Technological University in Houghton, MI and graduated with a B.S. in Civil Engineering. As a civil engineer she worked as an inspector for the Washington State Department of Transportation and in construction management for Skanska USA. She decided to combine her entrepreneurial skills with her passion for knitting, natural fibers, and color and opened Sleeping Dragon Yarn, a hand-painted yarn company selling yarns both wholesale and retail. She successfully ran this business until deciding to move to Raleigh, NC to pursue a Masters of Science degree at North Carolina State University in Textile Engineering.
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Chapter 1. Introduction

Eco-friendliness has become an important consideration in the textile world. This is because certain synthetic dyes and the auxiliary chemicals employed can cause pollution issues. Consequently natural dyes are experiencing enhanced interest. In small niche markets, such as in parts of the craft industry, they have grown in demand. They are spreading into other markets as well, with the trend toward green and environmentally friendly products growing.

Cotton is widely used in the textile industry (1, 2). It typically requires the use of a mordant when dyeing with natural dyes. Metal salts are the most commonly used mordants, as they are able to form coordinating complexes that link dyes and cotton. A variety of metal salts are used as mordants (aluminum potassium sulfate, ferrous sulfate, copper sulfate, etc…), and the best one to use depends on the natural dye and the color desired. The mordant used can impact fastness properties and can result in different colors. For example, the use of ferrous sulfate results in darker more muted shades than when using alum (3–6). Natural dyes produce uncommon, soft, soothing shades that synthetic dyes have difficulty replicating. Key knowledge concerning practical uses of natural dye has been passed down through craft dyers (7–21) as opposed to academic and commercial institutions, however this is changing. Research involving extracting, mordanting, dyeing, and improving the fastness properties of natural dyes is getting more attention (3, 22–29). There is also investigation into new sources of natural dyes to create different shades and to expand sources. Since natural dyes are obtained from either plants or animals they are potentially renewable
resources but not necessarily available in large quantities all the time or available in all areas of the world.

Chapter 2. Literature Review and Background

2.1. Cellulosic Fibers

2.1.1. General Information

Cellulose is the most abundant natural organic polymer. For thousands of years it has been indispensable as a material for clothing and housing (2) as well as a useful raw material for many industrial products. The textile industry uses cellulosic materials such as cotton, flax, hemp, jute, and regenerated cellulosic fibers including rayon, Tencel, and Lyocell (1).

The cotton plant belongs to the natural order of the MALVACEAE or the mallow family and the genus of Gossypium. Various species of Gossypium are grown in subtropical climates and have their own unique properties such as staple length, strength, elongation at break, uniformity ratio, fineness (micronaire), color, and trash content. Each cotton fiber is a unicellular hair collected from the seed of the cotton plant. Each fiber, however long or short, consists of one single complete vegetable cell. Cotton fibers are structured into concentric zones with a hollow central core known as the lumen. The outermost layer is known as the cuticle and is a thin film of fats, pectins, and waxes. Under the cuticle is the primary wall composed mainly of cellulose with fibrils arranged in a crisscross pattern. Further into the center is the secondary wall composed of cellulose, which constitutes the bulk of the fiber (30). (Figure 1)
2.1.2. Structure

There have been several proposed structures for cellulose (C₆H₁₀O₅). The most widely accepted molecular structure is the linear polymer, $\beta(1\rightarrow4)$ linked D-glucosyl residues as seen in Figure 2. Cotton is a cellulosic (92-97% cellulose) natural fiber with –CH₂OH and –CHOH groups as the main chemical functionality (β). Cellulose forms a ribbon like structure capable of bending and twisting due to the oxygen bridges connecting

![Molecular structure of the repeating unit of cellulose.](image)

Figure 2. Molecular structure of the repeating unit of cellulose.
the glucose rings. Six hydroxyl groups are attached to each cellobiose repeat unit in the chain. These form intermolecular hydrogen bonding which aids the stability of the molecule. The degree of polymerization for cellulose depends on the source (wood pulp, cotton, plant fibers, etc.) and can be as low as 200 for regenerated cellulose from wood pulp and as high as 10,000 for natural cellulose based fibers such as cotton (2).

2.1.3. Cotton and Natural Dyes

Most natural dyes either form coordinating complexes with metal ions (mordants) or form hydrogen bonds with the appropriate functional groups of the fiber substrate, in this case the cellulose chain. The washfastness of dyed textiles to different conditions depends on many factors. Among the most important factors is the type of dye-fiber or dye-fiber-mordant interactions/bonds, as well as the chemical structure and substituent groups of particular colorants (3). While cotton is the most used natural textile fiber, due to its durability, low price, ease of washing, and comfort while wearing, is difficult to dye with natural dyes because it absorbs a lower amount of colorant in comparison to other natural fabrics such as wool. Animal fibers are less hydrophilic, which allow for better fixation of natural dyes (4).

2.2. Natural Dyes for Cellulose

2.2.1. History

Natural colorants obtained from plants and animals have been used since prehistoric times. In 1856 with the accidental synthesis of mauveine by Perkins in England and its
subsequent commercialization, synthetic dyes began to compete with natural dyes. As more synthetic dyes became available, there was a rapid decline in the use of natural dyes. This was due to the availability of a wider range of possible colors with better color fastness at a reasonable price. However, research has shown that certain synthetic dyes can produce compounds that are allergic and carcinogenic, making them detrimental to human health and the environment (31–46). This has led to an increase in interest in natural dyes, believing them to be more environmentally friendly and more biodegradable than synthetic ones (5, 23, 47).

A few companies produce natural dyes commercially. Robbia in Milan produces water extracts of natural dyes such as weld, chlorophyll, logwood and cochineal, under the Eco-Tex certifying system, and supplies the textile industry. Allegro Natural Dyes in the USA produces natural dyes under the Ecolor label for the textile industry. They produce dyes such as indigo, ossage orange, madder, cutch, and cochineal. They report an 85% exhaustion level and claim to have developed a mordant based on a non-toxic aluminum formulation and biodegradable auxiliary substances. Bleu de Pastel, in Lectoure, France sells an extract of woad leaves. Natural dyes are available for craft use in the UK from Fibrecraft, which offers, along with other textile craft supplies, a wide range of natural (and synthetic) dyes in small quantities. There are also several small textile companies using natural dyes. The Natural Dyeing Company, in the UK, produces hand-knitted sweaters dyed with natural dyes on a commercial basis. Renaissance Dyeing produces rugs, shawls, embroidery yarns, and special commissioned products, all dyed with natural dyes (48).
2.2.2. Advantages and Disadvantages

Nearly all dyes are currently produced from synthetic compounds. This has greatly reduced the costs while enhancing certain application and wear characteristics. Yet, practitioners of the craft of natural dyeing maintain that natural dyes have superior aesthetic qualities while commercial practitioners believe that natural dyes are non-viable on the grounds of quality and economics. In the Western world, natural dyeing is predominantly practiced only as a handcraft while synthetic dyes are used in most commercial applications (47). Natural dyes produce very uncommon, soothing, and soft shades compared to their synthetic counterparts. In contrast, synthetic dyes, while available in a wide range of colors at economical prices, include examples that have been known to cause skin allergy and other harm to the human body, employ hazardous compounds during synthesis, and release toxic chemicals to the environment (31–46). In order to have successful commercial use of natural dyes for a particular fiber, the appropriate techniques and standards for dyeing need to be established and adopted. By developing scientific techniques and procedures, new shades with acceptable colorfast behavior and reproducible color yield can be achieved (5).

Natural dyes do have some interesting advantages over synthetic dyes. The shades natural dyes produce are usually soft and lustrous. They can produce a wide range of colors by a mix and match system (matching different natural dyes with different mordants). A small variation in the dyeing technique or the use of different mordants with the same dye (polygenetic type natural dye) can shift the colors by a wide range or create totally new colors. This is not as easily possible with synthetic dyes. Natural dyes are usually a renewable resource since many are agro-renewable/vegetable based and also biodegradable.
Many synthetic dyes are made from petro chemicals and thus not renewable. The effluents associated with use of natural dyes can be less toxic than that of synthetic dyes (49). In some cases the waste in the by-product of natural dyes becomes an ideal fertilizer for use in agricultural fields (harda, indigo, etc.). Many natural dye plants also thrive on wastelands where other crops cannot be grown. Plants containing dyes like madder grow as hosts in tea gardens so there is no additional cost or effort required in growing them. Use of natural dyes is a more labor intensive industry that could in turn provide job opportunities for those engaged in cultivation, extraction, and application of these dyes on textiles, food, leather, etc. The application of natural dyes also has the potential to earn carbon credit by reducing consumption of petroleum based synthetic dyes. Some natural dyes are known anti-allergens, anti-oxidants, anti-inflammatoryatories, and some are currently undergoing research as supplements and/or treatments for various medical conditions (50). Others are thought to have UV-protection and antimicrobial properties (51). Some natural dyes are enhanced with age, while synthetic dyes fade with time. Natural dyes bleed but do not generally stain other fabrics. They are usually moth proof and can replace synthetic dyes in kids’ garments and foodstuffs safely (4, 5).

Unfortunately, not all natural dyes are eco-friendly. Heavy metals and other potentially toxic substances may be present in some natural dyes and their mordants, therefore natural dyes require testing for toxicity prior to use (52–54). Natural dyes do have other limitations. It is difficult to reproduce shades, as natural dyes are agro-products that vary from one crop season to the next, from one location to the next, and with maturity period (50, 55). This makes it difficult to standardize a recipe as the natural dyeing process
and its color development depend not only on color component but also on the materials. Dyeing with natural dyes is also a more expensive process, as it requires skilled workmanship to dye. The low color yield of some sources of natural dye necessitates a large volume of dyes, more mordant materials, and longer times for dyeing and mordanting processes. Some naturally dyed textiles may change color when exposed to the sun, sweat, and air. Mordants can pose problems for effluent disposal since they do not completely exhaust from the aqueous medium. Most natural dyes are fugitive even when applied in conjunction with a mordant and thus their color fastness performance ratings are often low.

There are a limited number of dyes leading to a limited range of shades available. Availability is also an issue. Not all sources for natural dyes can be cultivated in all areas around the world and as an agricultural commodity they are subject to weather, drought, and natural disasters. Additionally there are limitations on the fabrics that can be dyed with natural dyes. Natural fibers such as wool, silk, linen, and cotton work best with natural dyes and synthetic fibers are more challenging (4, 5).

2.2.3. Classification

Natural dyes have been classified in various ways over the years. Early methods of classification were based on the alphabetical arrangement of dyes. Later, methods were developed based on chemical structure, their origin or source, application method, and color. In the Color Index, natural dyes are classified according to their chemical composition as well as their major applications. Natural dyes make up a separate section in the Color Index where they are arranged according to hue within their application category (4, 5).
Natural dyes classified on the basis of their chemical structure fall into a wide range of classes. Indigoids, anthraquinonoids, alpha-naphthaquinones, flavonoids, flavones, dihydropyrans, anthocyanidins, and carotenoids are the main classes (4). Indigoids produce blue or purple with indigo and tyrian purple the most commonly known. Almost all red natural dyes are based on the anthraquinone structure; madder, lacs, and cochineal are some examples. Flavonoids yield yellow dyes and can be classified under flavones, isoflavones, aurones, and chalcones. Most of the natural yellows are derived from hydroxyl and methoxy substituted flavones and isoflavones. A common example is weld. Alpha-naphthaquinones are generally disperse dyes and give shades of orange. The most common example is henna. Generally they are cultivated in India and Egypt. Dihydropyrans are closely related to the flavones in chemical structure and are substituted dihydropyrans, such as haematin and its leuco form haematoylin. These give dark shades on silk, wool and cotton. Logwood, brazil wood and sappan-wood are common examples of plants sources of haematoylin. Anthocyanidins give a substantive orange dye for wool and cotton. Carajurin is a naturally occurring member of this class. Finally carotenoids also provide a orange pigment due to the presence of long conjugated double bonds (4, 5). The structures of these natural dyes are seen in Table 1.
Table 1. Examples of natural dyes and their molecular structures.

<table>
<thead>
<tr>
<th>Natural Dye</th>
<th>Structure</th>
<th>Natural Dye</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indigo (^a)</td>
<td><img src="image" alt="Indigo Structure" /></td>
<td>Weld (Luteolin) (^f)</td>
<td><img src="image" alt="Weld Structure" /></td>
</tr>
<tr>
<td>C(<em>{16}H</em>{10}N_2O_2)</td>
<td></td>
<td>C(<em>{15}H</em>{10}O_6)</td>
<td></td>
</tr>
<tr>
<td>Tyrian Purple (^b)</td>
<td><img src="image" alt="Tyrian Purple Structure" /></td>
<td>Henna (Lawsone) (^g)</td>
<td><img src="image" alt="Henna Structure" /></td>
</tr>
<tr>
<td>C(_{16}H_8Br_2N_2O_2)</td>
<td></td>
<td>C(_{10}H_6O_3)</td>
<td></td>
</tr>
<tr>
<td>Madder (^c)</td>
<td><img src="image" alt="Madder Structure" /></td>
<td>Logwood (Haematoxylin) (^h)</td>
<td><img src="image" alt="Logwood Structure" /></td>
</tr>
<tr>
<td>(Alizarin) C(_{14}H_8O_4)</td>
<td></td>
<td>C(<em>{16}H</em>{14}O_6)</td>
<td></td>
</tr>
<tr>
<td>Lacs (^d)</td>
<td><img src="image" alt="Lacs Structure" /></td>
<td>Carajurin (^i) (Anthocyanidin)</td>
<td><img src="image" alt="Carajurin Structure" /></td>
</tr>
<tr>
<td>(Laccaic Acid) C(<em>{20}H</em>{14}O_{10})</td>
<td></td>
<td>C(<em>{15}H</em>{11}O)</td>
<td></td>
</tr>
<tr>
<td>Cochineal (^e)</td>
<td><img src="image" alt="Cochineal Structure" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Carminic Acid) C(<em>{22}H</em>{20}O_{13})</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

See references \(^a\)(4, 5, 56) \(^b\)(4, 5, 56) \(^c\)(4, 5, 56) \(^d\)(4, 5, 56) \(^e\)(4, 5, 56) \(^f\)(4, 5, 56) \(^g\)(4, 5, 56) \(^h\)(4, 5, 56) \(^i\)(4, 5, 56)

Another common method of classification of natural dyes is according to source. Based on their source or origin natural dyes can be grouped into three classes: vegetable sources, insect or animal sources, and mineral sources. Vegetable dyes are obtained from the
various parts of plants and herbs. These typically produce distinct pale to dark shades on both natural and synthetic fibers. Dyes obtained from insect or animal sources are usually based on the anthraquinone structure and produce some of the most important red dyes, such as cochineal. These dyes usually have good lightfast properties and they combine well with metal salts to form metal-complex dyes with good washfastness.

Classification of natural dyes by method of application has also been popular. Natural dyes are classified either as substantive or non-substantive dyes. Natural substantive dyes can be further subdivided into substantive dyes for cotton (turmeric and pomegranate), substantive dyes for wool and silk (turmeric and pomegranate), acid dyes (saffron), and basic dyes (berberine). Natural substantive dyes for cotton can be applied to all natural fibers textiles but have the most affinity to cotton. They are applied from a boiling dye bath. Acid and basic dyes are generally applied to wool and silk and although basic dyes can also be used with tannic acid treated cotton. Acid dyes are applied from an acidic medium. The dye molecules contain sulfonic group(s) that can form an ionic bond with the ammonium group(s) of wool and silk. Basic dyes upon ionization give colored cations and form an ionic bond with the carboxylate group of wool and silk and are applied under neutral to mildly acidic conditions. Typically, they are posses poor lightfastness (5). Mordant dyes are equally suited to both animal (wool) and vegetable (cotton) fibers and have electron-donating groups capable of forming a complex with the transition metal salt. A few natural dyes also fall into the category of vat dyes. These dyes are insoluble in water and include indigo, woad, and Tyrian purple. They must be converted to their water-soluble form by reducing with sodium hydrosulfite and then solubilizing with alkali. Then, they can be applied to
fibers and the true color is produced by oxidation followed by treatment with oxygen in a hot soap solution (4).

2.2.4. Flavonoids

Flavonoids are derivatives of phenylalanine and the acetate coenzyme A esters. They are based on a C6-C3-C6 structure (4, 57). The C3 link forms a heterocyclic pyrone ring. The oxidation state of this ring and the hydroxylation or methoxylation of a ring (first C6 group) allows distinction between the various groups of flavonoids. Flavones, flavonols, and anthocyanidins are the three major groups of flavonoids and can be seen in Table 2 (4).

Table 2. Three major groups of flavonoids: flavone, flavonol, and anthocyanidin.

<table>
<thead>
<tr>
<th>Flavone</th>
<th>Flavonol</th>
<th>Anthocyanidin</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="" /></td>
<td><img src="image2" alt="" /></td>
<td><img src="image3" alt="" /></td>
</tr>
</tbody>
</table>

See references (4, 56) (4, 56) (4, 56)

Anthocyanidins are the most highly colored of the flavonoids and yield blue, bluish red, mauve, brown, orange, and reddish brown pigments. The degree of hydroxylation or methoxylation of the B ring (the second C6 group) determines the color given. Specifically, hydroxylation increases the blueness and methoxylation increases the redness (4).
Flavones and flavonols (3-hydroxyflavones) are the main chromophores in the flavonoid natural yellow dyes. Most occur in plants as sugar derivatives (usually glycosides), which are hydrolyzed in the dyebath to the parent flavonoid. They require the use of mordants and bind to the metal via the carbonyl group and the adjacent phenol moiety (58). Flavones tend to be more permanent but paler in color where as flavonols tend to fade in strong light. Examples of particular interest are quercetin, rutin, and old fustic (morin, maclurin and kaempferol are the components of old fustic) and can be seen in Figure 3 and Figure 4. Flavones and flavonols are structurally derived from flavone (C_{15}H_{10}O_{2}) by the substitution of the hydrogen atoms in various ways and to varying degrees by hydroxyl or methoxyl groups (59).

![Quercetin](image1.png)

Quercetin \(^m\)
C_{15}H_{10}O_{7}

![Rutin](image2.png)

Rutin (C_{27}H_{30}O_{16}) \(^n\)

Figure 3. Molecular structures for quercetin and rutin.
See references \(^m(4, 56, 60) \)^n(4, 56, 60)
2.2.5. Extraction

Extraction refers to separating the desire color component from the source material physically with the aid of a solvent. Natural dyes of different origins can often be extracted using an aqueous medium with or without addition of salt/acid/alkali/alcohol. Methods include an extraction bath (6, 24, 26, 51, 55, 61–63), supercritical fluid extraction (64, 65), enzyme assisted extraction (66, 67), and alcohol/organic solvent (60, 68). Optimum extraction conditions are determined through extracting the color component from the source material while varying extraction parameters of solvent and measuring the optical density of corresponding colored liquor by using a spectrophotometer. Gravimetric yield of color can also be measured by filtering the extraction liquor through standard filtration processes followed by evaporation of solvent, washing, and drying to get the purified natural color.
To optimize the extraction method of the color component in aqueous medium, finely cut and dried source material of the natural dye is ground into powder form and the color component is extracted in water using a standard process. The aqueous extraction of dye liquor is carried out under varying conditions, such as temperature of the extraction bath, time of extraction, pH of extraction liquor, concentration of color source material, and material-to-liquor ratio (MLR). In each case, the optical density or absorbance at a particular (usually maximum) wavelength for the aqueous extract of the natural dye material is estimated using UV-VIS absorbance spectrophotometry (5).

2.2.6. Isolation and Characterization

There has been a growing interest in flavonoids, over the last few years, due to the wide range of biological effects they are capable of exerting. These effects include anti-inflammatory, anti-allergic, anti-bacterial properties as well as antioxidants and free radical scavengers. Mass spectrometric techniques are well suited to analysis of flavonoids since they can provide significant structural information on small quantities of pure samples as well as mixtures. Liquid chromatography coupled to mass spectrometry (LC-MS) represents a powerful tool in the analysis of natural products. The mass spectrometer is a universal detector that can achieve very high sensitivity and provide information on the molecular mass and on structural features. Sample preparation, while depending on the sample type, is fairly simple owing to the high selectivity of mass spectrometry. Flavonoid samples can be prepared by homogenization, liquid extraction and filtration and/or centrifugation but since
they are generally part of a complex mixture, a purification step is necessary (69, 70). This can be accomplished by dry column chromatography.

Dry column chromatography, in conjunction with thin-layer chromatography, is an effective method to purify or separate mixtures of organic compounds. Fractions obtained with dry columns are directly related to those obtained on thin-layer chromatographic plates. This explicit relationship is important because it allows the direct transfer of conditions from a thin-layer plate to a “dry-column.” Another advantage of this technique is that the nylon column is transparent to ultraviolet light. This makes it particularly useful for compounds that show up better under ultraviolet light region of the spectrum rather than in the visible light region of the spectrum. In addition, once the development of the column has been completed, the components can be isolated simply by sectioning the column with a knife and extracting each section with the appropriate solvent (71). This technique has been used with synthetic dyes (72), and it yields results consistent with other forms of column chromatography (73).

High-performance liquid chromatography (HPLC) is a chromatographic method in which high pressure is used to force solvent through closed columns containing very fine particles to achieve high-resolution separations. These systems are comprised of a solvent delivery system, a sample injection valve, a high-pressure column, a detector, and a computer to control the system and display the results. The columns are steel or plastic, 5 to 30 cm long with a diameter of 1 to 5 mm. Inside the column is the stationary phase, highly pure, spherical, microporous particles of silica, which is permeable to solvent and has a surface area of several hundred square meters per gram. In adsorption chromatography, the solvent
molecules vie with the solute molecules for sites on the stationary phase. The relative abilities of different solvents to displace a given solute from the adsorbent are almost independent of the nature of the solute. Elution occurs when the solvent molecules displaces solute molecules from the stationary phase. The measure of solvent adsorption energy is the eluent strength. The more polar a solvent, the great an eluent strength it has for adsorption chromatography. The greater the eluent strength, the faster the solutes will elute from the column. Adsorption chromatography on bare silica is a case of normal-phase chromatography, in which a polar stationary phase is used with a less polar solvent. A gradient elution is beneficial when one solvent does not provide suitably fast elution of all the components. In this case, increasing amounts of solvent B is added to solvent A to create a continuous gradient. HPLC columns are capable of providing narrow, symmetric peaks on chromatograms (74).

Mass spectrometry (MS) is a technique used to study the masses of atoms, molecules, or fragments of molecules. In order to obtain a mass spectrum, gaseous molecules or species desorbed from condensed phases are ionized, then the ions are accelerated by an electric field, and separated according to their mass-to-charge ratio. The display of the detector response versus the mass to charge ratio is the mass spectrum. Ideally, the area under each peak in the mass spectrum is proportional to the abundance of each isotope. A time-of-flight mass spectrometer separates the ions with the same kinetic energy but different mass to charge ratios, because the heavier ions require more time to travel a fixed distance. Mass spectrometry is often used as the detector in chromatography to proved qualitative and quantitative information. The spectrometer allows for high selectivity of the analyte of
interest. Liquid chromatography introduces a slight problem because the liquid from the column creates a large volume of gas when it is vaporized at the interface between the column and the mass spectrometer. This problem can be solved by using a pneumatically assisted electrospray or ion spray. The liquid from the column enters the steel nebulizer capillary along with a coaxial flow of N₂ gas. In positive ion mass spectrometry, the nebulizer is held at 0 volts and the spray chamber is held at -3 500 V. Where as in negative ion mass spectrometry, all voltages are reversed. The strong electric field at the nebulizer outlet combined with the coaxial flow of N₂ gas, creates a fine aerosol of charge particles. In order to look for specific compounds, selected ion monitoring is useful. In this case the mass spectrometer is set to monitor just a few mass to charge ratios. By knowing the mass to charge ratio of specific compounds, it is possible to determine if they are present in the analyte (74).

HPLC and LC-MS have been used extensively for analysis of natural dyes, components of natural dyes, and even to determine nicotine content. HPLC-MS of flavonoids of natural dyes has been investigated in both negative and positive ion modes to develop an optimized method. Using pure standards it was found that the signal intensity of the precursor ion in the negative ion mode was approximately ten times lower than that observed in the positive ion mode. Since formic acid was added to the mobile phase for efficient HPLC separation, this makes sense as formic acid can lower the ionization efficiency of the compounds in the negative ion mode. However, it was found that the performance of the column was significantly diminished when formic acid was removed from the mobile phase, broad peaks were observed and resolution between some peaks was
lost. Further investigation found that despite the addition of the formic acid to the chromatographic mobile phase, the most sensitive and selective was negative electron ion mode for dye identification (75). Positive and negative electrospray ionization coupled with liquid chromatography in tandem with mass spectrometry and diode-array detection has been used for determination of phenols in rose hip extract. The identification was based on comparison with product ion spectra of commercial standards. This was determined to be very useful in identification of some phenols (quercetin for example) in extracted rose hip. It was shown that consistently negative ion mode yielded clearer peaks than positive ion mode for these compounds (69). LC-MS in negative electrospray mode was also successfully used to identify flavonol glycosides such as rutin in noni fruit powder (76). LC-MS has been used in positive ion mode to determine nicotine content in allergenic extracts of tobacco leaf (77) and also in analysis of hair to evaluate tobacco smoke exposure (78).

2.2.7. Mordants

Natural dyes generally require that the materials to be dyed are mordanted with a metallic salt or with the addition of a metallic salt to the dyebath. In their pure state most natural dyes are lightly colored and produce poor results when used alone (4,79).

Mordants are typically metallic salts used to create an affinity between the fiber and the dye. The main objective of the mordant is to open up the pores so that the colorant can penetrate the fibers thus aiding in the fixation of the dyes to the substrate. Mordants can also be used with dyes, which may be applied directly. In this case, their function is to form an insoluble compound with the dye and the fiber itself. This improves the fastness properties
of the dyed material. Mordants are generally classified into three categories: metal salts, tannins and tannic acid, and oil mordants. Cotton can be treated with these mordants to acquire an affinity for basic dyes (4, 79).

In the past naturally occurring metal salts (aluminum obtained from different species of clubmoss from Finland and Scotland and iron from surface scum or bottom sediment of bogs as well as mineral sources) were used as mordants to ensure the fastness of color to sunlight and washing (58). Today, metal salts of aluminum, chromium, iron, copper, and tin are also used. Some of the important metallic mordants are alum, potassium dichromate, ferrous sulphate, copper sulphate, stannous chloride, and stannic chloride (4). Transition metal ions typically have strong coordinating power and/or are capable of forming weak to medium attraction/interaction forces that act as bridging materials creating substantivity when a textile material is impregnated with such metallic salts and is subjected to dyeing with different natural dyes. The mordantable groups facilitate fixation of the dye. These mordants combine with dye in the fiber and form an insoluble precipitate causing both the dye and the mordant to be fixed into the textile with a reasonable level of washfastness (5). Tannic acid and oil mordants can also act as primary mordants for metal salts. For instance, cotton mordanted with tannic acid is able to absorb all types of metallic mordant and can be readily dyed with mordant dyes. In such cases, the metallic mordant forms complexes with the carboxyl groups of the tannic acid (4).

Generally, there are two subcategories of metallic mordants, brightening and dulling. Brightening mordants include alum, chrome (potassium dichromate), and tin. Alum is cheap, easily available, and safe to use. It typically produces pale versions of the prevailing dye
color in the plant. Chrome is also referred to as red chrome and is more expensive. \( \text{Cr}^{+3} \) and \( \text{Cr}^{+6} \) are considered harmful for human skin and objectionable heavy metals beyond a certain limited amount. As such their use has been limited per norms of eco-standards. They are also sensitive to light in solution and therefore change color under light exposure. Tin \( (\text{SnCl}_2) \) yields brighter colors than any other mordant. However, it oxidizes upon exposure to air and may impart a stiff hand to the fabric. Copper and iron are known as the dulling mordants. Copper \( (\text{CuSO}_4) \) is readily soluble in water and easy to apply. It gives some special effects in shades which otherwise cannot be obtained. However, it is also limited under eco-standard norms as an objectionable heavy metal beyond certain limits. Iron \( (\text{FeSO}_4) \) is also readily soluble in water. It is used for darkening, browning, or blackening colors/shades. It is easily available and one of the oldest mordants known. It is used extensively to get grey to black shades (5).

Tannins and tannic acid (Figure 5) are important to processes using natural dyes to produce yellow, brown, grey, and black colors. They are also known to improve the affinity of dyes to certain fibers. They are mainly used in the preservation of leather, as glues, stains, and mordants. Vegetable tannins are bitter and astringent substances occurring as excretions in bark, leaves, fruit, galls, etc. of plants. These excretions maybe used directly or in concentrated form. Tannins are naturally occurring compounds of high molecular weight (500 to 3000). The phenolic hydroxyl group allows them to form effective cross-links between proteins and other macromolecules. Tannins which contain ortho-dihydroxyl groups can form chelates, which yield different colors with depending on the metals (4, 79). Besides these reactions, it is postulated that tannins form three types of bonds with proteins.
Figure 5. Molecular structure of tannic acid (4, 56, 79)

(wool and silk) and cellulose (cotton, rayon, viscose, etc.). Hydrogen bonds can form between the phenolic hydroxyl groups of the tannins and the free amino and amido groups of the protein, or the hydroxyl groups of the other polymers. Ionic bonds can form between suitably charged anionic groups on the tannin and cationic groups on the protein. Finally, covalent bonds can form by the interaction of any quinone or semiquinone groups that may be present in the tannins and suitably reactive groups in the protein or other polymers.

Stability of the tannin-fiber bond depends on the pH, ionic strength and metal chelators (79).

Numerous tannin-containing substances are used as mordants to dye textiles. Vegetable tannins are divided into two distinct classes hydrolyzable and condensed. The class is determined according to the phenolic nuclei involved and how they are joined.
Hydrolyzable tannins contain a polyhydric alcohol, such as glucose as the core. The hydroxyl groups of this alcohol are esterified either partially or wholly by gallic acid or its cogener. These tannins are readily hydrolyzed by acids, bases, or enzymes to create carbohydrates and a number of isolable crystalline phenolic acids. The other acid isolated from these tannins is ellagic acid. When left to stand for a long period of time, an aqueous solution of tannic acid decomposes by fermentation, which can be inhibited by adding boric acid. An alkaline solution of tannic acid rapidly absorbs oxygen from the atmosphere and turns brown due to decomposition. Condensed tannins contain only phenolic nuclei. When treated with a hydrolytic reagent, particularly in an acid solution, they show a tendency to polymerize into insoluble, amorphous red colored compounds known as phlobaphenes. These tannins are formed by the condensation of two or more molecules of flavon-3-ols, specifically catechin (4).

Oil mordants are predominantly used in the dyeing processes using madder to produce Turkey red. The main objective of the oil mordant is to form a complex with alum, which is used as the principal mordant. Alum is soluble in water and does not have much affinity for cotton so it is easily washed out of treated fabrics. The naturally occurring oils contain fatty acids such as palmitic, stearic, oleic and ricinoleic acid, and their glycerides. The -COOH groups of the fatty acids react with metal salts and are converted into -COOM (M denotes the metal). Sulfonated oils (concentrated sulfonic acid treated with oils) have better metal binding capacity than natural oils. This is due to the introduction of the sulfonic acid group -SO₃H. This reacts with metal salts to produce –SO₃M. The bound metal can then
form a complex with a mordant dye such as madder, producing a Turkey red color with superior fastness and hue (4).

2.2.8. Mordant Application

Since metallic mordants are soluble in water, this means that they are only loosely held by cotton fibers and are easily washed out. Therefore, they must be precipitated onto the fabric by one of two methods. Mordants can be converted to an insoluble form once they are in the fibers or the fibers can be treated with oil or tannic acid followed by impregnation of the treated fabric with a mordant solution. In the latter method the oil or tannic acid works to fix the metallic mordant to the cotton (4).

Mordanting can be achieved in a few different ways. Fabric can be pre-mordanted (before dyeing), simultaneously mordanted and dyed, or maybe mordanted post dyeing. In pre-mordanting methods, the textile substrate is treated in an aqueous solution of the mordant for an optimized period of time at an optimized temperature with a mordant liquor ratio of 1:5 to 1:40 and then dried with or without washing. The mordanted textile is then dyed, following an optimized dyeing procedure that may require salt, soda ash or acid depending on the type of textile substrate and the type of natural dye (4).

In the simultaneous mordanting and dyeing system, the textile substrate is immersed in a dye bath containing both the mordant and the dye in a definite quantity and dyeing may be started once optimum conditions are reached. This method may also require that auxiliaries depend on the textile and natural dye used. To maximize color yield, optimized
conditions process variables can be studied for specific fiber-mordant-natural dye systems (4).

The post-mordanting method involves the dyeing process carried out for bleached textiles in the absence of a mordant at pre-determined dyeing conditions. The dyed fabric is treated in a separate bath called a saturator containing the mordant solution. Treatment conditions may vary depending on the type of fiber, dye, and mordant (4).

Generally there are a few guidelines to determine which mordant and mordant method to use. Pre-mordanting and post mordanting using ferrous sulfate and aluminum sulfate improve the color uptake and color retention upon repeated washing. Alum and aluminum sulfate used have almost no environmental toxicity and are easily available. The use of copper or ferrous sulfate yields high lightfastness whereas stannous chloride or alum do not (4).

2.2.9. Dye Application

Modern dyeing processes and machinery in the textile industry have been designed for use with synthetic dyes. Synthetic dyes have been specifically designed to give good results on both synthetic and natural fibers while most natural dyes do not perform well on synthetic fibers. There have been some studies involving the application of natural dyes to natural and synthetic textiles (80–82) using modern dyeing techniques, such as open bath (80, 81), high temperature high pressure (HTHP) (80, 81), and padding techniques (83, 84). In these methods, the amount of dye used to produce a desired shade is less than in conventional methods for natural dyes. Other studies have shown that reuse of the dyebath
during dyeing with natural dyes not only substantially decreases the amount of color in
effluents, but also offers savings in dye cost and energy (85). The reuse of dyebaths shows
consistency in shades, indicating that it may be possible to achieve effluent free dyeing with
natural dyes. Another way to improve color depth involves the multi-dip method (86). This
results in much deeper shades that could not be achieved through any of the single dip
(exhaust) methods (4).

In craft dyeing, the dyes are extracted from the plants by soaking in water, alkali,
alcohol, or another organic solvent. Heat is sometimes required and for some dyes a
multistage process is needed. Alternatively, the fiber may be dyed with the whole plant and
then treated with a mordant to change the color of one of the dyes, as in the case of dyeing
with walnut husks. Craft dyeing often uses the vat dyeing process, such as when dyeing with
indigo (7).

The production of textiles requires the use of large volumes of water and it is
estimated that the dyeing process alone can use as much as 70% of the total water
consumption involved. Since most natural dyes need to be extracted with water and usually
in conjunction with heat, it is not clear how the current commercial machinery and dyeing
processes need to be adapted to use with natural dyes (4).

Three different methods are used to dye textiles on a commercial scale. In the first
method the material to be dyed is held stationary while the dye liquor moves through it, such
as in raw stock or package-machine dyeing. In the second method, the textile material is
moved through the dye liquor, such as in chain warp dyeing, jig dyeing, beck dyeing, and
continuous dyeing. This method is used for most woven materials except for stretch fabrics.
These are both exhaust dyeing methods. In the third method, the textile material and the dye liquor both move, such as in the Klander-Weldon skein dye machine and jet dyeing (4).

### 2.2.10. Fastness Properties

Generally, natural dyes do not have the same fastness properties as those exhibited by synthetic dyes (87). Mordanting and after treatments help improve fastness properties but the intrinsic susceptibility of the chromophore of natural dye colorants to photochemical degradation generally results in poor fastness to washing and light (52).

The resistance of a material to change its color characteristics (fading) due to environmental conditions or treatment like washing, dry cleaning, or exposure to different agency heat or light is known as color fastness. Fading is the change in the color with or without loss of depth of shade for exposure to particular environment, agent, or treatments either by lightening or darkening of the shades. Bleeding is the transfer of color to a secondary material in contact accompanying white fiber material of similar or dissimilar nature. Color fastness is usually rated either by loss of depth of color or color change in the original sample or it is expressed by a staining scale, meaning that the accompanying material gets tinted or stained by color from the original fabric. Crockfastness (rubbing) indicates how well the dye is embedded into the fabric or if the color can be removed by rubbing the surface of the dyed fabric. The crockfastness of most natural dyes has been found to be moderate to good and does not require additional after treatment (4, 5).

A number of factors affect the lightfastness and washfastness ratings of natural dyes. Factors affecting lightfastness include the chemical structure of the colorant, concentration of
the dyes, nature of the fiber (wool, silk, cotton, etc), and choice of mordants (4, 29). The choice of mordant in particular has a marked effect on lightfastness. For example, the use of alum alone to mordant cotton then dye with natural dyes does not lead to as good washfastness as cotton mordanted with tannic acid, then alum, then dyed (88). The washfastness of some cottons dyed with natural dyes without use of a mordant were low to medium, and use of a mordant resulted in a significant improvement in dye adsorption and washfastness (28, 52). The key factor affecting washfastness is the strength of the bond between natural dye and fiber, as change in hue due to the breaking of the dye-metal complex during washing, and ionization of natural dyes during alkaline washing can occur. The washfastness of some natural dyes can be improved by a post-treatment with alum or a dye-fixing agent resulting in the formation of a dye-fiber complex or a cross-link between the dye and fiber (4, 29). Using a sodium phosphate fixative after treatment can increase the washfastness of may yellow natural dyes particularly those that contain flavonoids (7).

Vegetable yellow dyes generally are known to have low tinctorial value and the shades are pale. Consequently, they fade more quickly depending on the mordant used. Aluminum and tin mordants tend to allow more light fading than chrome, iron, or copper.

Flavonoids constitute a major class of natural yellow dyes. The basic flavonoid chromophore is susceptible to photochemical attack and probably leads to the formation of quinones. As a result, the yellow color turns to a dull brown as commonly seen in old textiles in museums. As an example, flavonic colorants present in weld, sandalwood, and plums exhibit a lightfastness rating which is much higher than that of the flavonol colorants, such as those in
quercetin, Persian berries, and onion skin. This is due to the presence of a highly photosensitive hydroxyl group in position 3 in flavonic colorants (4).

K/S and CIE L*a*b* values can be used to analyze the changes in color resulting from washfastness and lightfastness testing. The Kubelka-Munk model is commonly used in color assessment. It allows the prediction of spectral reflectance for a mixture of components (colorants) as a function of absorption, K, and scattering, S, coefficients. The Kubelka-Munk coefficients K and S are related to, but not equal to, the fundamental optical coefficients for light absorption and scattering. The strength of any colorant is related to the absorption property. Reflectance, not absorption, is measured and it is known that when reflectance increases, absorption decreases (89). In opaque systems, reflectance is transformed to the ratio of absorption, K, to scattering, S, (K/S), known as “K over S.” For materials where the colorants have negligible scattering properties in comparison to those of their supporting medium, like textiles, only the K/S ratio is used to characterize a colorant, leading to the expression “single-constant Kubelka-Munk theory.” For opaque materials, Kubelka and Munk found that internal reflectance, R_{λ,i}, depended on absorption K_{λ} and scattering, S_{λ}. Reversing this equation gives the well-known relationship between (K/S)_{λ} and R_{λ,i} when reflectance is scaled between zero and unity (90).

\[
(K/S)_{λ} = \frac{(1 - R_{λ,i})^2}{2 R_{λ,i}}
\]

The color strength can be determined using several methods. The lowest value of R (corresponding to the maximum value of absorbance) can be found and used to obtain K/S values of the sample and the standard then compute the strength. This is generally accepted and more or less agrees with visual observation. In commercial software packages, the
program automatically does it. Commercial software packages can also use an integrated wavelength approach for computation of color strength. In this instance, color strength was calculated at each wavelength and the average is taken as real strength of the colorant (89).

The color difference space is a three-dimensional space with approximately uniform visual spacing in terms of color difference judgments. CIELAB differences correlate with visual differences everywhere throughout the three-dimensional space. The difference in color between two samples can be quantified by plotting their coordinates in CIELAB. The distance between the two positions is defined by $\Delta E_{ab}^*$. Colors are often plotted omitting the $L^*$ dimension. Colors can be described by their redness/greenness ($a^*$), yellow/blueness ($b^*$), hue, and chroma. CIELAB is a rectangular coordinate system with axes of $L^*$, $a^*$, and $b^*$. The differences between a standard and a sample in lightness/darkness is $\Delta L^*$, redness-greenness is $\Delta a^*$, and yellowness-blueness is $\Delta b^*$. By convention, positive differences means that the batch has more of the variable than the standard.

$$\Delta L^* = L^*_{\text{batch}} - L^*_{\text{standard}}$$
$$\Delta a^* = a^*_{\text{batch}} - a^*_{\text{standard}}$$
$$\Delta b^* = b^*_{\text{batch}} - a^*_{\text{standard}}$$

The total color difference between two samples is the slant (Euclidean) distance in CIELAB.

$$\Delta E_{ab}^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

The differences in color are also defined by the differences in lightness ($\Delta L^*$). A lightness difference greater than zero means that the batch is lighter than the standard and a lightness difference less than zero means the batch is darker than the standard (90).

$$\Delta L^* = L^*_{\text{batch}} - L^*_{\text{standard}}$$
2.3. Tobacco

2.3.1. General Information

Tobacco has been used for a wide variety of purposes throughout the world, especially for smoking. While it has many harmful effects on the health of those who smoke it, including lung cancer, pulmonary diseases, bad breath, discolored teeth, decreased ability to taste and smell, gingival recession, tooth loss, oral leukoplakia, and increased risk of cancer in the mouth and gums, certain constituents have potential medicinal value. It is a known source of medicinally useful alkaloids and flavonoids (60).

While tobacco is a controversial crop, it is also a significant economic crop. It provides a livelihood for millions of people, and is a billion dollar industry. The most common tobacco produced in the world is *Nicotiana tabacum*. The only other species (*N. rustica*) is used on a limited commercial scale (91). Tobacco is further classified by major types and general uses. Scientists have found tobacco to be rewarding biological material to study. Most basic plant science knowledge was generated from studying tobacco plants (92–94). Some significant examples are photoperiodism, genetics and breeding, growth regulators, pollutants, viruses, plant nutrition, organic metabolism, photosynthesis and photorespiration, post harvest physiology, and biotechnology. There is a wealth of information concerning tobacco and still continues to be studied (91).

2.3.2. Colorant Components

Rutin (Figure 3), a well-known, natural antioxidant, is one flavonoid that has been found in tobacco. It makes up to 1% of the whole dried tobacco plant. Rutin can be used to
reduce capillary fragility, swelling, and bruising, improving micro-vascular blood flow, it
induces cAMP synthesis, inhibits phospholipase, and super oxidase, inhibits secretion and
aggregation of platelets, to name a few uses. Rutin has also been used as a coloring agent
and food additive in various food preparations, drinks, and even cosmetics. It has been
shown that rutin comprises 1.5% of unfermented tobacco leaves and 0.6% of waste tobacco
leaves when percolated using 70% methanol (MeOH) and 0.5% in fermented leaves (60).

Quercetin, another flavonoid, has also been found in extracts from waste tobacco
leaves (60, 95). Quercetin is found in a variety of food products and plants, including fruits,
seeds, vegetables, tea, coffee, and has been used in natural dyes. It frequently occurs as
glycosides; e.g., rutin in which the hydrogen of the R-4 hydroxyl group is replaced by a
disaccharide. Quercetin is termed the sugarless form of rutin (96).

2.3.3. Nicotine

Nicotine is the major alkaloid component of commercial tobacco. It comprises
approximately 95% of the total alkaloid content and 1.5% by weight in commercial tobacco.
While tobacco does contain other alkaloids with toxic properties (nornicotine and anabasine)
they are present in much smaller amounts, making up 3% or less of the alkaloid content (97,
98). Nicotine (Figure 6) is not a direct cause of most diseases related to tobacco but it is
highly addictive which in turn causes exposure to a diverse array of carcinogens and other
bioactive compounds in tobacco. Nicotine based medications are also widely used as
nicotine replacement therapies and are also studied as experimental therapies for Parkinson’s
disease, Alzheimer’s disease, and ulcerative colitis (98).
Freshly distilled nicotine (C_{10}H_{14}N_{2}) is a colorless and nearly odorless liquid. Its boiling point is 247°C and its melting point is -79°C. It is miscible with water in all proportions below 60°C and above 210°C, with volume contraction, and is soluble in organic solvents. Upon exposure to air, nicotine darkens, becomes more viscous, and develops and unpleasant fishy odor (97).

The health related aspects of cigarette smoking have generated much of the attention and interest in the effects of nicotine on humans and animals. There is no doubt about nicotine’s potent toxicity to humans. As nicotine enters the body by inhalation, it is quickly distributed through the bloodstream and is capable of crossing the blood-brain barrier. In approximately seven seconds nicotine reaches the brain. The amount of nicotine inhaled in tobacco smoke (1 to 2 mg per cigarette) is a fraction of the amount contained in tobacco leaves, as most of the nicotine is destroyed in burning of the cigarette. When chewing tobacco, however the amount of nicotine released into the body tends to be much greater. It is generally agreed that 30 to 60 mg of nicotine is a lethal dose for an adult. This makes
nicotine a potentially deadly substance. It is more toxic than alkaloids such as cocaine, which has a lethal dose level of 1000 mg (97, 98).

2.4. Related Studies

The renewed interest in using natural dyes is contributing to more attention to natural dyes in academic research. While most practical information for use of natural dyes and mordants is found in the craft industry (8–21, 99) there has been a recent rise in the attention given to them in formal research and investigation into the science of how they work and how improvements can be made.

Using various traditional natural dyes with different mordants to compare color strength and fastness properties provided a starting point for most research pertaining to natural dyes. Dyeing of organic cotton with madder root, walnut shell, henna, horse chestnut, pomegranate peel, bereris vulgaris root, thyme, and sage tea using different mordants (copper sulfate, aluminum potassium sulfate, potassium tartrate, and citric acid) was studied using pre-mordanting methods. It was found that the color strength of the natural dye used depended on the mordant and generally a medium to high level of wash fastness could be obtained and crock fastness was highly rated (6).

Studies have been conducted on dyeing cotton and jute with an aqueous extract of tea. The dyeings were performed with and without the use of metal salts (ferrous sulfate heptahydrate, aluminum potassium sulfate dodecahydrate, and copper sulfate pentahydrate) as mordants. Three different dyeing methods were used: pre-mordanting, simultaneous mordanting, and post-mordanting. The resulting wash and lightfastness properties were
examined in terms of K/S and CIELAB. Medium shades (K/S = 3.9) were obtained on jute in acidic media, while under identical dyeing conditions white cotton fabrics could be dyed in light depths (K/S=2.0) (100).

The colorfastness and deodorizing properties of cotton, silk, and wool dyed with coffee sludge extract have been explored. Aqueous extractions from Coffea Arabica L. (90°C for 90 min) were then used to dye cotton, silk, and wool fabric using a variety of mordants (FeSO₄, CuSO₄, SnSO₄, MnSO₄, ZnSO₄, Al₂(SO₄)₃, NiSO₄, and CoSO₄) to compare color strength (K/S and CIELAB values), washfastness, lightfastness, and perspiration fastness. The resulting dyed cotton, wool, and silk fabrics were assessed for deodorization performance (61).

Application of aqueous extract of Eucalyptus to cotton has been explored. This work focused on optimization of extraction conditions by varying temperature and stirring times. Sodium sulfate was used during dyeing to improve exhaustion. Once the optimum temperature of the dye bath was established, the concentration of sodium sulfate was varied. Once the optimum sodium sulfate concentration was determined the dyeing time was varied. Washfastness, lightfastness, and crock fastness were then assessed. The dye obtained gave moderate saturation levels on cotton with medium to good fastness properties (62).

The influence of mordant concentration on dyeing cotton fabric with cochineal extract has been investigated. This work included color fastness to acids and alkalis; hot water and domestic washings, artificial light, and crock fastness were also assessed. It was found that a higher concentration of metal ions in the mordant solution resulted in better
color fixation and a deeper red hue of the dyed fabric. It was also found that hydrogen ion levels influenced red dye intensity (23).

Studies on color strength related parameters and compatibility along with color fastness properties when dyeing cotton fabrics with binary mixtures of jackfruit wood and other natural dyes have been undertaken. Varying proportions of binary mixtures of aqueous extracted jackfruit wood with other natural dyes (manjistha, red sandlewood, marigold, sappan wood, and babool) were used to dye cotton pre-mordanted with myrobolan (20%) and aluminum sulfate successively. The fabrics were then evaluated for color strength (K/S) coefficient of variation, brightness index, changes in hue (ΔH), and total color difference (ΔE). It was found that jackfruit wood in combination with manjistha had the highest degree of compatibility. Assessment of light, washing, and crocking resulted in moderate to poor washfastness and light fastness and good crock fastness. To improve wash and light fastness, the dyed fabrics were treated with cationic dye-fixing agents and a UV-absorber (3, 22).

Dyeing cotton, wool, and silk with *Hibiscus mutabilis* (Gulzuba) gave very good fastness properties to all fabrics tested. Use of aqueous extracts of Gulzuba flowers to dye cotton, silk and wool fabrics pre-treated with 2 to 4% mordants (alum, stannic chloride, stannous chloride, and ferrous sulfate) using a liquor ratio of 1:40 gave very good fastness properties on all fabrics (24).

An investigation into the application of natural dyes derived from the Acacia plant family by padding of cotton fabric and using three different mordanting techniques was performed. Generally, it was found that a change in the mordant resulted in a different shade using the same dye. Copper sulfate (optimum concentration of 15 g/L) produced beige
shades while ferrous sulfate (5 g/L) produced grey. Post-mordanting yielded better depth of shade than either pre- or meta- mordanting (84).

Canadian golden rod has been used to assess the quality of different crops of plant material collected over a five-year period of time on consistency in dyeing. Aqueous solutions containing extracted flavonoids were characterized using direct photometry and absorbance measurements. While the correlation between the photometric results and the color depth of the dyeings was not sufficient to allow characterization of the plant material with regards to the final dyeing, relatively small differences in color depth and shade were found in the plant material collected (55).

Rubia cordifolia (tamin) leaves, stems and roots, produce an anthraquinone reddish orange dye. Use of a plant based biomordant, Nausankhee, instead of a metal salt in conjunction with the Rubia cordifolia has been explored. The use of the biomordant enhanced dyeability due to the aluminum content in the leaves and enhanced the fastness properties. This could provide an even more environmentally friendly mordant option. Use of commercial sonicator dyeing also indicates that this process could be scaled up for commercial use with minimum changes to equipment employed with synthetic dyes (25).

The sonicator method of dyeing was also used to dye cotton fabric with Eclipta (an annual herb having leaves rich in natural dyes) and the results compared to those using conventional natural dye application methods. Higher color strength values were found using the sonicator method, indicating this is a method of dyeing that merits further investigation with natural dyes. The time/dye uptake data revealed enhanced dye uptake.
compared to conventional methods and fastness properties of the sonicator dyed fabrics were rated fair to good. Unfortunately, the “conventional” dye method was not specified (26).

Natural colorant material extracted from Crocus sativus (saffron flowers) has also been explored as a dye for cotton. The factors affecting dye uptake such as pH, salt concentration, mordant (aluminum potassium sulfate, copper sulfate, stannous chloride, ferrous sulfate, and tannic acid), temperature, time in dyebath, as well as use of an ultrasonic dye bath were investigated for impact on color strength (K/S and CIELAB) and fastness properties (light, wash, and perspiration). The ultrasonic method gave better color strength than traditional dyeing methods. While different mordants and mordant methods (pre-, post-, and simultaneous) produced a wide range of hues, pre-mordanting showed higher color strength (63).

It has been found that dyeing cotton knits with certain natural dyes using metallic salt mordants adds UV-protection and antimicrobial functionality to the fabrics. The performance properties of these dyeings were influenced by the fabric structure, the type and concentration of mordant, the class and dye extract percent, and the mordanting- dyeing sequence. Pre-mordanting with metal salts improved K/S and UV-protection. The antibacterial activity depended on the type of mordant (zinc-chloride). The nature of the extracted dye had no significant effect on UV-protection or antimicrobial properties. These functionalities were also shown to persist after up to 20 cycles (51).

Generally, the light fastness properties of natural dyes are poor. There has been research in an effort to improve light fastness for commercial use. Wool specimens pre-mordanted with alum, chromium, copper, iron, or tin were dyed with 18 yellow natural dyes.
Those samples were then exposed to a xenon-arc lamp for varying AATCC Fading Units (5, 10, 20, 40, and 80). The color changes were evaluated by CIELAB and gray scale classification. Turmeric, fustic, and marigold based dyes faded more than the yellow dyes derived from cherry leaves, goldenrod, or onion. Dyes applied with tin and alum mordants faded more than dyes mordanted with chrome, copper, and iron. The mordant was found to affect the light fastness more than either the dye or the length of exposure to light (88).

Another approach to enhancing the light fastness of natural dyes has involved the addition of a UV absorber after treatment. Wool and silk were dyed with goldthread, amur cork tree, gromwell, and redwood after mordanting with iron sulfate, aluminum acetate, potassium dichromate, and copper acetate. Fabric samples were then treated with two UV-absorbers and subjected to a xenon arc lamp for 20 h. This treatment was shown to improve lightfastness on both wool and silk without affecting color or shade (101).

Cotton also was evaluated in a similar manner. Madder, weld, and woad were used to dye cotton then antioxidants and UV absorbers were applied. In all cases, improved lightfastness was observed (29). However, mordants did not appear to be used with these dyes. It is likely that the addition of pre-mordanting would provide additional lightfastness.

High-performance liquid chromatography (HPLC) analysis of indigoids on wool has been conducted. Comparative colorimetric analysis was performed via reflectance spectrophotometry on standard multifiber swatches containing 13 different synthetic and natural fiber materials. Each swatch was dyed with a different indigoid then colorimetric properties such as K/S, UV-VIS, and CIELAB values were measured and compared. From this information excellent functional correlations were observed among these properties and
it is thought that these relationships will be applicable to similar dyeing on other fabrics. Wool showed the highest color strength while cotton the poorest, which is consistent the general performance of natural dyes on wool versus cotton (102).

There has been an extensive review on the use of mass spectrometry in the structural analysis of flavonoids. In this study, the use of mass spectrometry in combination with other spectroscopic techniques such as UV and NMR, or directly coupled in an LC UV MS NMR system were determined the most powerful technique in the identification of complex unknown compounds of plant origin (70).

2.5. Purpose

The interest in green products has been growing over the past few years and many countries have issued stricter environmental regulations on pollution in response to public awareness and concern about pollution. This has impacted the textile coloration industry, which has been one of the major producers of effluents containing dyes and associated chemicals (42, 46). While a general theme of energy and water consumption savings in textile processing with continual improvements in the efficiency of dyeing processes aimed at minimizing environmental impact has been emphasized in the textile industry for the past decade (50), another approach has involved examining the dyes and chemicals used.

The use of tobacco for smoking in the USA has been declining and alternative uses for this important agricultural product would provide economic outlets to this industry. The colorants present in tobacco have the potential for dyeing textiles. Interestingly, an appreciable amount of tobacco dust is generated as a waste product from the processing of
cured tobacco into cigarettes. This material has no apparent use and is usually thrown away. Consequently, the objective of this project was to develop methods for isolating, characterizing, and utilizing colorants derived from extracting commercial tobacco dust.

Chapter 3. Experimental

3.1. Materials

This study was conducted with three samples (T-1, T-2, T-3) of tobacco dust generated during the processing of cured tobacco into cigarettes, as supplied by Philip Morris (Altria Group, Inc.) located in Richmond, VA. T-1 (ID number 00662235) was a medium brown color, T-2 (ID number 00655742) was a light golden brown color, and T-3 (ID number 00536914) was a dark brown color. A photograph of the tobacco dust varieties can be seen in Figure 7.

![Tobacco dust samples](image)

Figure 7. Tobacco dust samples used in this study. Top left: T-1, top right: T-2, and bottom center: T-3.

Two fabrics used in mordanting and dyeing experiments, cotton (100%) interlock and cotton (100%) knit, were obtained from HanesBrands in Winston-Salem, NC. Both fabrics had been scoured and bleached.
Solvents used in extractions were HPLC grade isopropyl alcohol (IPA), HPLC grade de-ionized water, HPLC grade acetonitrile, formic acid, de-ionized water, and n-hexane. Mordants used were aluminum sulfate octadecahydrate (Al$_2$(SO$_4$)$_3$ •18H$_2$O), ferrous sulfate heptahydrate (FeSO$_4$•7H$_2$O), tannic acid, and soda ash. Triton X-100 surfactant and de-ionized water from Siemens system were used in stock solution preparation. Salt treatment experiments included in the dyeing studies used sodium sulfate. The direct dye fixative experiments used cationic fixative Sera Fast C-TE. The DMDHEU after treatment experiments included Freerez 980 reactant, Freesoft BUN softener, non-ionic wetting agent Wetaid, and catalyst MG-2. Sodium dihydrogen phosphate monohydrate (NaH$_2$PO$_4$•H$_2$O) was used in the sodium phosphate fixative experiments. Table 3 indicates purity levels and suppliers of chemicals used.
Table 3. Purity levels and suppliers of chemicals used in experiments.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Purity</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>De-ionized water</td>
<td></td>
<td>Siemens system</td>
</tr>
<tr>
<td>IPA</td>
<td>HPLC grade</td>
<td>FisherScientific</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>High resolution gas chromatography</td>
<td>FisherScientific</td>
</tr>
<tr>
<td>Al₂(SO₄)₃</td>
<td>Certified ACS crystalline</td>
<td>FisherScientific</td>
</tr>
<tr>
<td>FeSO₄</td>
<td>Certified ACS crystalline</td>
<td>Fisher Chemical</td>
</tr>
<tr>
<td>Tannic acid (C₇₆H₅₂O₄₆)</td>
<td>95%</td>
<td>Acros Organics</td>
</tr>
<tr>
<td>Soda ash (Na₂CO₃)</td>
<td></td>
<td>Brenntag</td>
</tr>
<tr>
<td>Triton X-100 surfactant</td>
<td>Electrophoresis grade</td>
<td>FisherScientific</td>
</tr>
<tr>
<td>Sodium sulfate</td>
<td>Anhydrous 97%</td>
<td>Arcos Organics</td>
</tr>
<tr>
<td>Cationic fixative Sera Fast C-TE</td>
<td></td>
<td>DyStar</td>
</tr>
<tr>
<td>Freerez 980 reactant</td>
<td></td>
<td>Noveon</td>
</tr>
<tr>
<td>Freesoft BUN softener</td>
<td></td>
<td>Noveon</td>
</tr>
<tr>
<td>Non-ionic wetting agent (Wetaid)</td>
<td></td>
<td>Noveon</td>
</tr>
<tr>
<td>Catalyst MG-2</td>
<td></td>
<td>Noveon</td>
</tr>
<tr>
<td>Sodium biphosphate monobasic</td>
<td>USP/FCC Monohydrate</td>
<td>FisherScientific</td>
</tr>
<tr>
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<td>HPLC grade</td>
<td>Burdick &amp; Jackson</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>HPLC grade</td>
<td>Burdick &amp; Jackson</td>
</tr>
<tr>
<td>Formic Acid</td>
<td>For mass spectrometry 98%</td>
<td>Fluka</td>
</tr>
</tbody>
</table>

3.2. Instruments

Accelerated solvent extractions were performed using a Dionex ASE 350 Accelerated Solvent Extractor equipped with 10-mL and 22-mL cells. The pH of mordanting and dye bath solutions was measured using a Thermo Electron Corporation Orion 3 Star portable pH meter equipped with a VWR Symphony combination electrode.

Ahiba Nuance, Mathis Labomat, Mathis Padder, and Mathis JFL 12997 machines, General Signa Company Lindberg/Blue M Stable Therm electric oven, Yomato mechanical
convection, and Precision Screen Machines conveyor oven were used for mordanting and dyeing studies.

High performance liquid chromatography (HPLC) analysis was performed with a Waters 2996 Photodiode array detector and Alliance Waters 2695 separations module. HPLC-mass spectrometry (LC-MS) was performed using an Agilent 1200SL HPLC coupled to an Agilent Technologies 6520 Accurate-Mass-Quadrupole Time-of-flight (Q-TOF) instrument equipped with Agilent Qualitative Analysis B.04 software. UV-visible spectrophotometry (UV-VIS) was performed using an Agilent Technologies Cary 300 UV-VIS Spectrophotometer coupled with Cary Win UV software. Atomic Absorption analysis (AA) was performed with Perkins Elmer AAnalyst 300 and Win Lab 3.5 software after digestion of samples in a CEM MARS microwave digester. The X-rite Color i7 bench-top spectrophotometer with Color iMatch Professional software was used to measure K/S and L*a*b* values.

3.3. Bench-top Tobacco Extractions

3.3.1. Water Extraction

Tobacco dust (100 g) was placed in a 1-L Erlenmeyer flask. The dust was covered with water (600 mL) and aluminum foil was placed over the flask opening. The mixture was heated to a gentle boil on a hot plate, underneath a hood, and held at the boil for either 60 or 120 min. A clean 1-L beaker was weighed and the aqueous extract was decanted into the beaker. The water was then evaporated over low heat using a hot plate. The residue was allowed to cool and weighed. The percent yield of extracted material was calculated.
3.3.2. Isopropyl Alcohol Extraction

Tobacco dust (100 g) was transferred to a 1-L Erlenmeyer flask. The dust was covered with IPA (300 mL) and aluminum foil was placed over the flask opening. The mixture was heated to a gentle boil on a hot plate, underneath a hood, and held at the boil for either 60 or 120 min. A clean 1-L beaker was weighed and the extract was decanted into the beaker. The IPA was evaporated over low heat using a hot plate. The residue was allowed to cool and weighed. The percent yield of extracted material was calculated.

3.4. Accelerated Solvent Extractor Procedure using 10-mL cells

Cells (10-mL) were filled with a mixture of 0.70 g tobacco dust and 9.0 g 0.75 mm glass beads. Three-min extraction cycles were used for 3 cycles, with a 25% rinse volume and a 60 s purge using deionized water as the solvent. Three different temperatures (80°C, 100°C, and 120°C) were used to determine the optimum temperature for tobacco dye extraction. In another set of experiments, the temperature was held constant at 80°C and the length of time of cycles was 3, 6, and 9 min to determine the optimum length of time for the cycles.

Three solvents, hexane, IPA, and de-ionized water, were also compared using this procedure. Each solvent was used for one 5-min cycle out of the three cycles per sample at 120°C for each solvent. The order of the solvents was hexane, IPA, then de-ionized water or de-ionized water, IPA, then hexane.

Solvents were evaporated using a rotary evaporator. Percent yield was calculated.
3.5. Accelerated Solvent Extractor Procedure using 22-mL Cells

Cells (22-mL) were filled with a mixture of approximately 3.0 g tobacco dust and approximately 4.0 g of 4 mm glass beads. Three 5-min cycles were used with a 25% rinse volume and a 60 s purge. Deionized water at 120°C was used as the solvent.

3.6. Mordant Application Procedures

3.6.1. Initial Mordant Procedure

3.6.1.1. Aluminum Sulfate

A solution (400 mL) of Al₂(SO₄)₃ (2% owb) was placed in a 500-mL Ahiba Nuance stainless steel canister. A dry 10-g piece of clean white 100% cotton knit fabric was added to the mordant bath and agitated for 1 min. Canisters were loaded into the Ahiba Nuance and heated to 80°C at a rate of 4°C/min, held at 80°C for 60 min, then cooled until they reached a minimum of 40°C at 25 rpm. Fabric was removed so it could be placed directly into the dye bath.

In a second set of experiments a 10% owf Al₂(SO₄)₃ bath was used for mordanting 10 g of 100% cotton knit. A bath (200 mL) was prepared in stainless steel canisters and the Mathis Labomat was used. The bath was heated to 80°C at 2°C/min and held there for 60 min, then cooled to 40°C. Samples were then laid horizontally on a rack in the Yomato mechanical convection oven DKN 810 in the Pilot Plant at 50°C until dry.
3.6.1.2. Ferrous Sulfate

A solution (400 mL) of FeSO$_4$ (2% owb) was prepared in a 500-mL Ahiba Nuance stainless steel canister. A dry 10-g piece of clean white cotton knit fabric was added to the mordant bath and agitated for 1 min. Canisters were loaded into the Ahiba Nuance and heated to 80°C at a rate of 4°C/min and held at 80°C for 60 min, then cooled until they reached a minimum of 40°C at 25 rpm. Fabric was removed so it could be placed directly into the dye bath.

In a second set of experiments 1% owf FeSO$_4$ for 10 g 100% cotton knit solution. A bath (200 mL) was prepared in stainless steel canisters and the Mathis Labomat was used. The bath was heated to 80°C at 2°C/min and held there for 60 min, then cooled to 40°C. Samples were then placed in the Yomato mechanical convection oven at 50°C until dry.

3.6.2. Pad Procedures

3.6.2.1. Aluminum Sulfate

Al$_2$(SO$_4$)$_3$ (10 g/100 mL) in water solution was prepared to achieve 10% owf. The mordant solution was applied with a padder to achieve 100% wet pick-up. After padding, samples were laid horizontally on a rack in a 45°C mechanical convection oven until dry.

3.6.2.2. Ferrous Sulfate

FeSO$_4$ (5 g/500 mL) in water solution was prepared to achieve 1% owf. The mordant solution was applied with a Mathis Padder to achieve 100% wet pick-up. After padding, samples laid horizontally on a rack in a 45°C mechanical convection oven until dry.
3.6.3. JFL Dyeing Procedures

3.6.3.1. Mordanting with Al₂(SO₄)₃

Cotton knit fabric (50 g) was measured, cut, sewn into a continuous loop, and placed in the JFL. A solution (1.5 L) of Al₂(SO₄)₃ (5g/L) was prepared and added to the JFL. The temperature of the bath was brought up to 80°C at a rate of 2°C/min and held for 60 min. The bath was dropped, cool tap water (1.5 L) was added, and fabric was rinsed for 1 min.

3.6.3.2. Mordanting with FeSO₄

Cotton knit fabric (50 g) was cut, sewn into a continuous loop, and placed in the JFL. A solution (1.5 L) of FeSO₄ (0.5 g/L) was prepared and added to the JFL. The temperature of the bath was brought up to 80°C at a rate of 2°C/min and held for 60 min. The bath was dropped, cool tap water (1.5 L) was added, and fabric was rinsed for 1 min. The bath was dropped in preparation for dyeing.

3.6.4. Tannic Acid with Aluminum Sulfate Mordanting

Six trials were run using variations of the mordant procedure, in an effort to obtain optimum results in the shortest amount of time (7). A summary of these trials is seen in Table 4. The following variables were explored: length of time the fabric was allowed to sit in the different mordant baths, temperature of the tannic acid mordant bath, and length of time fabric sat in the dye bath.

In all trials, the tannic acid mordant bath had a liquor ratio of 40:1. Tap water was heated to 60 to 80°C then tannic acid (6 g/100 g cotton fabric) was added and stirred to
dissolve. Fabric was added while bath was hot. In Trials 1 and 3-6 the bath was removed from heat and allowed to cool. In Trial 2 the temperature was held constant at 60 to 80°C for 30 min. Then baths were dropped and fabric was rinsed in cool tap water once.

The Al$_2$(SO$_4$)$_3$ and soda ash mordant bath also used a liquor ratio of 40:1. Tap water (10% volume of total bath volume) was heated in a beaker to 60 to 80°C and Al$_2$(SO$_4$)$_3$ (50 g/100 g cotton fabric) was added. Soda ash (6 g/100g cotton fabric) was dissolved in room temperature tap water (2.5% volume of total bath). Under a hood, the soda ash solution was slowly added to the Al$_2$ (SO$_4$)$_3$ solution. The combined solution was then diluted with tap water to achieve a 40:1 liquor ratio. Cotton fabric was added and agitated. Fabric was allowed to sit in the bath for 24 h in Trials 1 and 3-4, for 30 min in Trial 2, and 2 h for Trials 5 and 6. Fabric was removed, squeezed to remove excess water, and either dried in a 35°C oven for 24 h, dried on a conveyor oven, or dried in a 35°C oven. Fabric was cut into 10-g pieces and rinsed 2 to 3 more times prior to dyeing as described for the Mathis Labomat.

Upon completion of dye cycle, the fabric was allowed to remain in the dye bath either overnight or until cooled to 25°C. Fabric samples were removed, rinsed until water ran colorless, dried hanging in General Signa Company Lindberg/Blue M Stable Therm electric oven 35°C. Then fabric was treated as described in the sodium phosphate fixative treatment.

Reproducible methods for each of the six trials follows and a summary of the trial methods can be seen in Table 4.
**Mordant Trial 1**

Cotton knit fabric (50 g) was cut and tap water (2 L) was heated to 60 to 80°C. Tannic acid (3 g) was added and stirred to dissolve. The fabric was added, the bath was removed from heat, and the fabric was allowed to sit in the bath over night. The bath was dropped and the fabric was removed and rinsed in cool tap water once.

Tap water (200 mL) was heated to 60 to 80°C and Al$_2$(SO$_4$)$_3$ was dissolved into the heated water. Soda ash (3 g) was dissolved in room temperature tap water (50 mL). Under a hood, the soda ash solution was slowly added to the Al$_2$(SO$_4$)$_3$ solution. The combined solution was diluted with tap water to 2 L, fabric was added, agitated, and allowed to sit overnight. Fabric was removed, squeezed to remove excess water, and dried in a 35°C oven for 24 h. Fabric was cut into 10-g pieces and rinsed twice prior to dyeing as described for the Mathis Labomat procedure. After completion of dye cycle, the fabric remained in the dye bath overnight. The fabric samples were removed, rinsed until water ran colorless, and hung to dry in General Signa Company Lindberg/Blue M Stable Therm electric oven at 35°C. Then the fabric was treated as described in the sodium phosphate fixative procedure.

**Mordant Trial 2**

Cotton knit fabric (50 g) was cut. Tap water (2 L) was heated to 60 to 80°C. Tannic acid (3 g) was added and stirred to dissolve. Fabric was added and allowed to steep for 30 min while temperature was maintained. The bath was dropped and fabric was rinsed in cool tap water once.
Tap water (200 mL) was heated to 60 to 80°C. Al$_2$(SO$_4$)$_3$ (25 g) was dissolved into the heated water. Soda ash (3 g) was dissolved in room temperature tap water (50 mL).

Under a hood, the soda ash solution was slowly added to the Al$_2$(SO$_4$)$_3$ solution. The combined solution was diluted with tap water to 1.5 L, the fabric was added, agitated, and allowed to sit for 30 min. Fabric was removed, squeezed to remove excess water, and dried on Precision Screen Machines conveyor oven. Fabric was cut into 10-g pieces and rinsed twice prior to dyeing as described for the Mathis Labomat procedure. After completion of dye cycle, the fabric remained in the dye bath overnight. The fabric was removed, rinsed until water ran colorless, hung to dry in a convection oven at 35°C. Then the fabric was treated as described in the sodium phosphate fixative procedure.

**Mordant Trial 3**

Cotton knit fabric (50 g) was cut. Tap water (2 L) was heated to 60 to 80°C. Tannic acid (3 g) was added, stirred to dissolve, and removed from heat. Fabric was added and allowed to sit while temperature cooled to room temperature (2 h). The bath was dropped and fabric was rinsed in cool tap water once.

Tap water (200 mL) was heated to 60 to 80°C. Al$_2$(SO$_4$)$_3$ (25 g) was added and stirred to dissolve. Soda ash (3-g) was dissolved in room temperature tap water (50 mL). Under a hood, the soda ash solution was slowly added to the Al$_2$(SO$_4$)$_3$ solution. The combined solution was diluted with tap water to 2 L, fabric was added, agitated, and allowed to steep overnight. Fabric was removed, squeezed to remove excess water, and dried on the conveyor oven. Fabric was cut into 10-g pieces and rinsed twice prior to dyeing as described...
for the Mathis Labomat procedure. After completion of dye cycle, the fabric was allowed to remain in the dye bath overnight. Then fabric samples were removed, rinsed until water ran clear, hung to dry in a convection oven at 35°C. Then the fabric was treated as described in the sodium phosphate fixative procedure.

**Mordant Trial 4**

Cotton knit fabric (50 g) was cut and tap water (2 L) was heated to 60 to 80°C. Tannic acid (3 g) was added, stirred to dissolve, fabric was added, and allowed to sit while temperature of the bath was maintained for 2 h. The bath was dropped and fabric was rinsed in cool tap water once.

Tap water (200 mL) was heated to 60 to 80°C and Al₂(SO₄)₃ (25 g) was dissolved into the water. Soda ash (3 g) was dissolved in room temperature tap water (50 mL). Under a hood, the soda ash solution was slowly added to the Al₂(SO₄)₃ solution. The combined solution was diluted with tap water to 2 L, the fabric was added, agitated, and allowed to sit overnight. Fabric was removed, squeezed to remove excess water, and dried on conveyor oven. Fabric was cut into 10-g pieces and rinsed twice prior to dyeing as described for the Mathis Labomat procedure. After completion of dye cycle, the fabric was allowed to remain in the dye bath overnight. Then fabric was removed, rinsed until water ran colorless, and hung to dry in a convection oven at 35°C. Then the fabric was treated as described in the sodium phosphate fixative procedure.
Mordant Trial 5

Cotton knit fabric (50 g) was cut and tap water (2 L) was heated to 60 to 80°C. Tannic acid (3 g) was added, stirred to dissolve, and removed from heat. Fabric was added and allowed to sit while the temperature of the bath cooled to room temperature (2 h). The bath was dropped and fabric was rinsed in cool tap water once.

Al$_2$(SO$_4$)$_3$ (25 g) was dissolved into room temperature tap water (200 mL). Soda ash (3 g) was dissolved in room temperature tap water (50 mL). Under a hood, the soda ash solution was slowly added to the Al$_2$(SO$_4$)$_3$ solution. The combined solution was diluted with tap water to 1.5 L, the fabric was added, agitated, and allowed to steep 2 h. Fabric was removed, squeezed to remove excess water, and dried. Fabric was cut into 10-g pieces and rinsed twice prior to dyeing as described for the Mathis Labomat procedure. After completion of dye cycle, the fabric was allowed to sit in the dye bath overnight. Then fabric was removed, rinsed until water ran colorless, and hung to dry in a convection oven at 35°C. Then the fabric was treated as described in the sodium phosphate fixative procedure.

Mordant Trial 6

Cotton knit fabric (50 g) was cut. Tap water (2 L) was heated to 60 to 80°C. Tannic acid (3 g) was added, stirred to dissolve, and removed from heat. Fabric was added and allowed to sit while the temperature of the bath cooled to room temperature (2 h). The bath was dropped and fabric was rinsed in cool tap water once.

Al$_2$(SO$_4$)$_3$ (25 g) was dissolved into room temperature tap water (200 mL). Soda ash (3 g) was dissolved in room temperature tap water (50 mL). Under a hood, the soda ash
solution was slowly added to the $\text{Al}_2(\text{SO}_4)_3$ solution. The combined solution was diluted with tap water to 1.5 L, the fabric was added, agitated, and allowed to sit 2 h. The fabric was removed, squeezed to remove excess water, and dried on conveyor oven. Fabric was cut into 10-g pieces and rinsed twice prior to dyeing as described for the Mathis Labomat procedure. After completion of dye cycle and cooling dye bath temperature to 25°C the fabric samples were removed, rinsed until water ran colorless, hung to dry in a convection oven at 35°C. Then the fabric was treated as described in the sodium phosphate fixative procedure.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Tannic acid Bath</th>
<th>$\text{Al}_2\text{S(O}_4\text{)}_3$ and Soda ash Bath</th>
<th>Dye Cycle Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td>Removed from heat 24-h bath</td>
<td>24-h bath Dried at 35°C for 24 hr</td>
<td>24 h</td>
</tr>
<tr>
<td>Trial 2</td>
<td>Bath maintained at 70 ±10°C 30-min bath</td>
<td>30-min bath Dried in conveyor oven with high heat</td>
<td>24 h</td>
</tr>
<tr>
<td>Trial 3</td>
<td>Removed from heat 2-h bath</td>
<td>24-h bath Dried at 35°C for 24 hr</td>
<td>24 h</td>
</tr>
<tr>
<td>Trial 4</td>
<td>Bath maintained at 70 ±10°C 2-h bath</td>
<td>2-h bath Dried at 35°C for 24 hr</td>
<td>24 h</td>
</tr>
<tr>
<td>Trial 5</td>
<td>Removed from heat 2-h bath</td>
<td>2-h bath Dried at 35°C until dry</td>
<td>24 h</td>
</tr>
<tr>
<td>Trial 6</td>
<td>Removed from heat 2-h bath</td>
<td>2-h bath Dried at 35°C until dry Until cooled to 25°C 3 h</td>
<td></td>
</tr>
</tbody>
</table>
3.7. **Dye Preparation**

3.7.1. **Water Extracted Dye**

Stock solutions of tobacco dust extract were prepared at 1% w/w. A solution of dried tobacco extract (1g/100 mL of tap water) was prepared with aid of a sonicator. Stock solutions were stored in a refrigerator.

3.7.2. **Isopropyl Alcohol Extracted Dye**

Triton X-100 surfactant was used at a 1:1 mass ratio with tobacco extract to prepare a 1% IPA tobacco extracted stock solution. The Triton X-100 surfactant was dissolved in a minimum amount of water. IPA extracted tobacco extract and acetone were mixed in a 1:1 mass ratio in a separate beaker. The tobacco extract/acetone mixture was then added to the Triton X-100 and water mixture and stirred. The remaining water required to make the 1% stock solution was added and the combination was mixed with the use of a sonicator.

3.8. **Tobacco Dye Application Procedure**

3.8.1. **Ahiba Nuance**

Tobacco extract solution (1%) (200 mL) was placed in Ahiba Nuance stainless steel canisters. Fabric (10 g) was added and the canister was closed and placed in the dyeing machine. The bath was heated to 80°C at 4°C/min and held there for 60 min, then cooled to 40°C at 4°C/min. The fabric was laid horizontally on a rack in the mechanical convection oven at 50°C until dry.
3.8.2. Mathis Labomat

Tobacco dust extract solution (1%) (200 mL) was placed in Mathis Labomat stainless steel canisters. Fabric (10 g) was added and the canister was closed and placed in the dyeing machine. The bath were heated to 80°C at 2°C/min and held there for 60 min, then cooled to 25°C at 2°C/min. The fabric was then hung vertically in an electric oven at 35°C until dry.

3.8.3. JFL

Tobacco dust extract solution (1.5 L) was added to the JFL (Figure 8) immediately after completion of mordanting. The dye bath was heated to 80°C at a rate of 2°C/min and held for 60 min. Bath was dropped. Cool tap water (1.5 L) was added and fabric was rinsed for 5 min. Rinse was repeated until rinse water was colorless. Fabric was removed from the JFL and padded to remove excess water, then hung to dry in electric oven between 30 and 40°C.
3.9. Fixative Treatments

3.9.1. DMDHEU treatment

Fabric mordanted with FeSO₄ and dyed in the JFL were padded using the Mathis Padder containing a solution (1 L) of Freerez 980 reactant (5% on weight of bath), Freesoft BUN softener (4% owb), non-ionic wetting agent Wetaid NRW (0.25% owb), and catalyst MG-2 (40% owb) to 100% wpu. Fabric was then dried on conveyor oven then cured using a W Mathis AG THN 13389 oven for 2.5 min at 190°C.

3.9.2. Sodium sulfate Treatment

Fabric samples (10 g) were mordanted with Al₂(SO₄)₃ and FeSO₄. The water extracted tobacco stock solution was used to prepare a dye bath (200 mL) of 20% owf with a
liquor ratio of 20:1 in a 300-mL Mathis Labomat stainless steel canister. Na$_2$SO$_4$ (7.5 g/L) was added to the dye baths. The Mathis Labomat machine was used to dye the fabric samples. The baths were heated to 80°C at 2°C/min, held for 60 min, and cooled to 40°C. Samples were then placed in the mechanical convection oven at 50°C until they were dry.

3.9.3. Direct Dye Fixative Treatment

Fabric (10 g) mordanted using FeSO$_4$ and dyed according to the Ahiba Nuance dye procedure was placed in cationic fixative (DyStar Sera Fast C-TE) solution (4% owf) in 200-mL Mathis Labomat stainless steel canister. The canister was placed in the Mathis Labomat machine and heated to 49°C and held for 20 min. Fabric was cooled to 40°C at 4°C/min, removed from bath and laid horizontally to dry in mechanical convection oven.

Fabric (10 g) mordanted with FeSO$_4$ and dyed with Ahiba Nuance dyed using the Ahibac Nuance dyeing procedure was padded (with cationic fixative (DyStar Sera Fast C-TE) solution (40 g/L) at 100% wpu) using a Mathis Padder. Fabric was dried in a mechanical convection oven at 130°C.

3.9.4. Sodium Phosphate Fixative Procedure

Tap water (2 L) was heated to 60°C and sodium phosphate (NaH$_2$PO$_4$•H$_2$O) (7.4 g/L) was added. Fabric was added, agitated, and the container was removed from heat and allowed to sit for 30 min. The bath was dropped and the fabric was rinsed well in tap water and hung to dry in an electric oven at 35°C.
3.10. K/S and L*a*b* Data Collection

Fabrics from mordanting/dyeing studies and wash fastness and light fastness testing were analyzed using a spectrophotometer to obtain K/S and L*a*b* values using X-rite Color i7 bench-top spectrophotometer coupled with Color iMatch Professional software. The average K/S and L*a*b* values of four readings were recorded for each fabric sample (folded twice for 4 layers).

3.11. Fastness Testing Procedures

The following fastness tests were performed on the dyed fabric samples:

2. Color Fastness to Light – AATCC Test Method 16-2004

3.12. Analytical Procedures

3.12.1. UV-VIS

To determine the amount of colorant in the dyebath after dyeing (c), a standard Beer-Lambert Law calibration curve was developed using the T-1 extract. The dye solutions were made at the following concentrations: 1.866 mg/mL, 0.933 mg/mL, and 0.620 mg/mL. The absorbance of each solution was measured on the Agilent Technologies Cary 300 UV-VIS Spectrophotometer at 380 nm and linear regression was used to give a calibration model. The absorbances of the diluted residual dyebaths were measured at 380 nm and the Beer-Lambert Law regression model was used to determine the concentration of dye in each
solution. See Table 5 for dilution factors for each dye bath analyzed.

Table 5. Dilutions of dyebaths for UV-VIS analysis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-1 Pre-Dye Bath</td>
<td>10</td>
</tr>
<tr>
<td>T-2 Pre-Dye Bath</td>
<td>10</td>
</tr>
<tr>
<td>T-3 Pre-Dye Bath</td>
<td>10</td>
</tr>
<tr>
<td>T-1 Post Dye Bath</td>
<td>2</td>
</tr>
<tr>
<td>T-2 Post Dye Bath</td>
<td>2</td>
</tr>
<tr>
<td>T-3 Post Dye Bath</td>
<td>2</td>
</tr>
</tbody>
</table>

3.12.2. LC-Mass Spectrometry

3.12.2.1. Isolation and Characterization of Colorant Components

LC-MS was performed with ESI parameters of 350°C gas temperature, gas flow of 12.0 L/min, and 25 psi nebulizer. Positive ion mode was used. Fragmentor voltage was 110-v. Injection volume was 1.0-µL. Binary pump (Table 6) used 0.5-mL/min flow. Solvent A consisted of water containing 0.1% formic acid. Solvent B was acetonitrile containing 0.1% formic acid. Post time was 3 min and the column temperature was 45°C. The column used was a Poroshell 120EC-C18 2.5 µm (3.0 x 100 mm). This method was developed based on the HPLC-MS analysis of constituents in noni fruit powder (76).
Table 6. Binary pump conditions for LC-MS isolation and characterization of colorant components.

<table>
<thead>
<tr>
<th>Time</th>
<th>Solvent A:B Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>95:5</td>
</tr>
<tr>
<td>3</td>
<td>50:50</td>
</tr>
<tr>
<td>4</td>
<td>50:50</td>
</tr>
<tr>
<td>5</td>
<td>5:95</td>
</tr>
</tbody>
</table>

3.12.2.2. Nicotine Analysis

LC-MS was performed with ESI parameters of 350°C gas temperature, gas flow of 12.0 L/min, and 25 psi nebulizer. Positive ion mode was used. Fragmentor voltage was 110-v. Injection volume was 0.1-µL. Binary pump (Table 7) used 0.35-mL/min flow. Solvent A consisted of water containing 0.1% formic acid. Solvent B was acetonitrile containing 0.1% formic acid. Post time was 3 min and the column temperature was 40°C. The column used was a Waters X-Bridge BEH Amide 2.5 µm (3.0 x 100 mm column XP).

Table 7. Binary pump conditions for LC-MS analysis of nicotine.

<table>
<thead>
<tr>
<th>Time</th>
<th>Solvent A:B Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>95:5</td>
</tr>
<tr>
<td>3</td>
<td>50:50</td>
</tr>
<tr>
<td>4</td>
<td>50:50</td>
</tr>
<tr>
<td>5</td>
<td>5:95</td>
</tr>
</tbody>
</table>
3.12.3. High Performance Liquid Chromatography

HPLC was performed to collect peak absorbance data from 380-780 nm. All analyses used a gradient of 10% to 90% using methanol and water over 16 min. The column used was an X-Bridge 3.0 x 100 mm with particles (2.5µ) and 10-µL injections were used.

3.12.4. Atomic Absorption

Microwave digestion of cotton samples (0.2500 g) in 20 mL 50% HPLC grade nitric acid used 1200 W at 110% using a ramp of 15-min at 800 PSI, control temperature of 180°C, held for 20 min. Atomic absorption was used to determine the percent Fe absorbed by the cotton knit fabric under different treatment conditions. Two samples of cotton treated only with the mordant bath, one cotton sample treated with the mordant bath followed by the dye bath, and two samples of untreated cotton were used in these experiments.

3.12.5. Dry Column Chromatography

Dry column chromatography was used to separate the colorants in the T-1 tobacco extract prior to their characterization using LC-MS. The T-1 extract (0.2331 g) was dissolved in methanol (2 mL) and water (to cloud point) and placed on a 1-inch diameter 24-inch long column of nylon tubing packed with silica gel (Selecto Scientific with particle size 32-63 µm). The column was eluted to the end with 1:1 ethyl acetate: methanol and four bands were found to contain colored components. The bands were removed, extracted using methanol, and the solvent was evaporated. The residues were analyzed using LC-MS.
Chapter 4. Results and Discussion

4.1. Extractions

4.1.1. Bench-Top Extractions

Initial tobacco extractions were performed in heated Erlenmeyer flasks. Two solvents, IPA and H$_2$O, were used with three tobacco dust samples (T-1, T-2, and T-3) to find which solvent and dust gave the highest amount of extract by weight (% mass).

The data (Table 8) were analyzed using JMP software by fitting a full model for analysis including main factors, interactions, and blocks based on small probability values. The model was then narrowed to only the main factors involved in the experiments.

Table 8. Bench-top extraction data for tobacco dust variety, solvent, and extraction time.

<table>
<thead>
<tr>
<th>Tobacco Dust Variety</th>
<th>Solvent</th>
<th>Time</th>
<th>Yield (g)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-3</td>
<td>IPA</td>
<td>120</td>
<td>7.55</td>
<td>7.6%</td>
</tr>
<tr>
<td>T-1</td>
<td>IPA</td>
<td>60</td>
<td>2.40</td>
<td>2.4%</td>
</tr>
<tr>
<td>T-2</td>
<td>IPA</td>
<td>60</td>
<td>3.55</td>
<td>3.6%</td>
</tr>
<tr>
<td>T-2</td>
<td>H$_2$O</td>
<td>120</td>
<td>16.58</td>
<td>16.6%</td>
</tr>
<tr>
<td>T-3</td>
<td>IPA</td>
<td>60</td>
<td>5.82</td>
<td>5.8%</td>
</tr>
<tr>
<td>T-3</td>
<td>H$_2$O</td>
<td>120</td>
<td>26.85</td>
<td>26.9%</td>
</tr>
<tr>
<td>T-1</td>
<td>H$_2$O</td>
<td>120</td>
<td>14.66</td>
<td>14.7%</td>
</tr>
<tr>
<td>T-2</td>
<td>IPA</td>
<td>120</td>
<td>3.83</td>
<td>3.8%</td>
</tr>
<tr>
<td>T-2</td>
<td>H$_2$O</td>
<td>60</td>
<td>21.36</td>
<td>21.4%</td>
</tr>
<tr>
<td>T-1</td>
<td>H$_2$O</td>
<td>120</td>
<td>18.70</td>
<td>18.7%</td>
</tr>
<tr>
<td>T-3</td>
<td>H$_2$O</td>
<td>60</td>
<td>21.01</td>
<td>21.0%</td>
</tr>
</tbody>
</table>
Interactions and block effects were found to be insignificant and were removed from the model. Table 9 represents a summary of the model statistics. An $R^2$ value of 94.7% is large. It was interpreted that this model was able to explain 94% of the variation obtained in the data. For a small experimental design such as this, 94% is a good value. The analysis of variance is a test of the hypothesis that the factors do not have any effect on the extraction yields. The results of the analysis of variance are shown in Table 10. A small probability value, in this case 0.0001, suggests that the hypothesis must be rejected thus validating the significance of the suggested model.

Table 9. Summary of fit for initial bench-top extraction experiments.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>R Square</td>
<td>0.947262</td>
</tr>
<tr>
<td>R Square Adj</td>
<td>0.917126</td>
</tr>
<tr>
<td>Root Mean Square Error</td>
<td>2.457018</td>
</tr>
<tr>
<td>Mean of Response</td>
<td>12.26083</td>
</tr>
<tr>
<td>Observations (or Sum Wts)</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 10. Analysis of variance for initial bench-top extraction experiments.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>4</td>
<td>759.03793</td>
<td>189.759</td>
<td>31.4331</td>
</tr>
<tr>
<td>Error</td>
<td>7</td>
<td>42.25856</td>
<td>6.037</td>
<td>Prob &gt; F</td>
</tr>
<tr>
<td>C. Total</td>
<td>11</td>
<td>801.29649</td>
<td></td>
<td>0.0001*</td>
</tr>
</tbody>
</table>
The parameter estimates, shown in Table 11, suggested that the solvent was the only significant factor in this process. The tobacco dust sample and extraction time had very small effects.

Table 11. Parameter estimates for initial beaker extraction experiments.

| Term            | Estimate   | Std Error | t Ratio | Prob>|t| |
|-----------------|------------|-----------|---------|-----|---|
| Intercept       | 12.260833  | 0.70928   | 17.29   | <.0001* |
| Tobacco[T1]     | -2.115833  | 1.003073  | -2.11   | 0.0729 |
| Tobacco[T2]     | -0.930833  | 1.003073  | -0.93   | 0.3843 |
| Solvent[Water]  | 7.599167   | 0.70928   | 10.71   | <.0001* |
| Time(60,120)    | 0.794167   | 0.70928   | 1.12    | 0.2998 |

Figure 9. Prediction Profiler for initial beaker extraction experiments.
The prediction profile (Figure 9) of the extraction data for tobacco varieties (T-1, T-2, and T-3) appear different but the error associated with it (see overlapping error bars) is large and therefore, the nature of the tobacco dust sample is not shown to have significant effects. Solvent type does have a significant effect, with extraction yields considerably higher for extraction with water than with IPA. Extraction time (60 vs 120 min) had a very small effect on the final yield, but was not significant statistically or practically.

The best combination of factors, as predicted by the model suggests that the highest yield should be obtained by extracting tobacco dust T-3 with water for 120 min. The yield predicted was 23.7 g with an associated error of ±3.75 g. A second experimental run with the same combinations gave a yield of 26.85 g.

Essentially, the bench-top method was effective for extractions but it was difficult to repeat with precision. The temperature was difficult to maintain, as care had to be taken to maintain the solvent volume while simmering the tobacco dust in it, and it was easy for tobacco dust to get mixed into extracts during decanting. In retrospect, a filtration step added prior to evaporating off the solvent could have helped keep tobacco dust solids out of the extract and given a more accurate assessment of the mass of extracted colorant. Other studies have employed similar methods for extraction (55), but the present method employed a shorter time compared to the 3 to 4 h period some typically reported (24, 26). Most studies used water as the solvent, with a few using solvents such as methanol or ethanol (60, 68).
4.1.2. Extracts by Accelerated Solvent Extractor (ASE)

Extraction cycles using three 3-min cycles and three 6-min cycles provided a better yield than three 9-min cycles as shown in Figure 10. While the 6-min cycles provided more extract than the 3-min cycles the difference was small and was within the margin of error. Based on this, three 5-min cycles were used as the optimized method. Figure 11 shows an extract obtained using the optimized method then evaporated.

![Extracts by Accelerated Solvent Extractor (ASE)](image)

Figure 10. ASE tobacco dye extraction yields as a function of time, using H₂O as the solvent.
Figure 11. ASE tobacco dust extract.

Figure 12 compares the extract yields achieved at 80, 100, and 120°C. Extractions run at 80°C and 100°C provided nearly the same extract amount. Extractions run at 120°C provided approximately 5% more tobacco extract. This experiment was repeated and all three temperatures provided tobacco extract yields within 1.5% of each other. This indicated that there was little benefit in going beyond 80°C for the aqueous extractions. To maximize the quantity of extract for dyeing studies it was decided to use 120°C in the optimized method.
Figure 12. ASE tobacco dye extraction yields as a function of extraction temperature, using H$_2$O as the solvent.

ASE was also used to compare yields using three different solvents (hexane, IPA, and H$_2$O) as shown in Figure 13. ASE cells were filed with a known mass of T-1 tobacco dust and glass beads then extracted using a different solvent for each of the three cycles. Cycle times remained at 5 min and the temperature was 120°C. The first three cells (1-3) were extracted with hexane first, then IPA, and finally H$_2$O. Then the order was reversed on the following two cells (4 and 5), to determine whether the order of the solvents used affected extraction yields.
Figure 13. Percent (%) yield of T-1 tobacco extracts using hexane, IPA, and H₂O, in one 5-min cycle.

Water afforded the most extract of the three solvents whether used at the beginning or end of the cycle and had a mean yield of 31.3%. IPA gave slightly higher yields when preceded by hexane rather than by water but with a mean yield of 10.1% the yield was too low to be practical. Hexane as the solvent resulted in very low extract yields. It did slightly better when it was used first than when it was used at the end of the cycle; but with a highest yield of 6% it is an impractical choice.

The number of 5-min cycles was also evaluated by comparing the percent yield of extract of each cycle using both IPA and water as solvents (Figure 14 and Figure 15). The temperature used was 120°C. In the data associated with water extracted T-1, cycle 1 shows widely varying data. The results of Trial 2, with 30% yield, was consistent with previous
Figure 14. Yield (%) of dye from water extracted T-1 in each of the three 5-min cycles.

Figure 15. Yield (%) of dye from IPA extracted T-1 in each of the three 5-min cycles.
results. It was evident that, the majority of the material extracted was obtained during the first cycle. The second cycle yielded about 10% extract. It was deemed worth while to run a second cycle to increase the yield by 10%. The third cycle yielded only 2 or 3% and was deemed unnecessary. Thus, in the optimized method only 2 cycles were used. In the IPA experiment (Figure 15), the majority of the material extracted was obtained in the first cycle. There was a discrepancy in the first trial where only 0.1% was extracted in the first cycle. However, all the data from this set of experiments was lower than the other two sets (trials 2 and 3). Given that trials 2 and 3 were more consistent with each other, trial 1 was considered an anomaly. This experiment reinforces that the yield from IPA extraction was too low to be considered practical. Visually, the H₂O extracts were brown, with those from the first extraction cycle the darkest. The second extract was a little lighter, and the third extract the lightest, although cycles 1 and 2 extracts were very close in color. The IPA extracts were generally more yellow and had a greenish tint. There was also less of a color difference in the extracts between extraction cycles.

In a final set of experiments, the optimum temperature for IPA extraction was examined, to determine if the percent yield of extract could be increased by using a lower temperature (80 and 100°C) during extraction for the three 5-min cycles. The results can be seen in Figure 16. The extraction at 100°C gave the highest yield (25%) and was promising for the continued use of IPA as a solvent for tobacco dust extraction.
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The ASE extractions were then upsized to 22-mL cells to determine if results so far would continue to be consistent and to determine the practicality of this extraction method for generating enough extract for dyeing studies. It was also necessary to determine the optimum ratio of tobacco dust to glass beads to maximize extract yield without clogging the ASE. Cells filled only with tobacco dust would not work in the ASE. The tobacco dust would pack tightly and not allow solvent containing the dye to drain from the cells. The glass beads dispersed in the tobacco dust allowed room for the solvent to come into contact with the tobacco dust, extract the colorant, and drain from the cell. Six experiments (Figure 17) were run using H$_2$O and IPA. For both solvents, two 5-min cycles were used and the temperatures used were 120°C for H$_2$O and 100°C for IPA. Figure 17 clearly shows that the 25% yield achieved by the IPA solvent extract in the previous experiment was not repeatable.
Figure 17. Comparison of % yield from H$_2$O and IPA extraction of tobacco dust T-1 using various ratios of tobacco dust to glass beads.

At this point it was clear that the use of IPA as a solvent for extraction was not practical and it was discontinued. The percent yield achieved by the various tobacco dust to glass bead ratios was consistent across the first four ratios then dropped off significantly, most likely due to the tobacco dust becoming too compact. The ratio 60:40 was chosen as the optimum ratio for use in running extractions. This method generated tobacco extract yields of 30 to 40% by mass. These yields were consistent with the yields from the bench-top extractions. Use of the ASE allowed extractions to be made much faster and easier.
4.2. Mordanting Studies

Three different mordants were employed in an effort to develop a procedure that would produce a desirable color, washfastness, lightfastness, and levelness on cotton when dyed with the tobacco dust extracts.

4.2.1. Ferrous Sulfate

The basic procedure for application of the mordant was consistent throughout these experiments. Changes in percent on weight of fabric (owf) of the mordant and the liquor ratio were the main factors examined. A liquor ratio of 40:1 with 80% FeSO₄ (owf) was used as the starting point. The mordant application procedure for the Ahiba Nuance was used and the fabric samples were transferred to the dye bath directly from the mordant bath. Upon examination of the fabric (very unlevel and showing signs of FeSO₄ migration during drying), the percent FeSO₄ (owf) was reduced to 20% and the liquor ratio was changed to 100:1. The method for the Ahiba Nuance was used but once the fabric was removed from the mordant bath it was rinsed in cool tap water and run through the padder to remove excess liquid. The percent FeSO₄ (owf) and the liquor ratio were too high to be scaled up for commercial use.

This led to the decision to use a padder to apply the mordant. It was also anticipated that this would help achieve a more level application. The FeSO₄ was applied at 3% (owf) this time, with a wet pick up of 100% and the fabric was used directly in the dyeing step. The percent FeSO₄ (owf) obtained was better, based on the appearance of the fabric. This experiment was repeated, but after padding the cotton was dried in a 45°C mechanical
convection oven then dyed. The percent owf FeSO₄ still seemed high based on the appearance of the fabric and it was reduced to 1% (owf) and wet pickup was 150%.

The fabrics mordanted and dyed using the Ahiba Nuance and Mathis Labomat were visually darker at the end of the dyeing cycle, indicating that they absorbed and retained more dye. Using the information gained from the padding experiments, 1% (owf) FeSO₄ was used, the liquor ratio was decreased to 20:1, and mordant was applied using the Mathis Labomat. The fabric was removed from the mordant bath and dried in a mechanical convection oven at 50°C. It was still difficult to achieve level samples at this stage and it was found that FeSO₄ itself turned the fabric orange-brown (Figure 18 and Figure 19).

The JFL was also used to apply the FeSO₄ mordant to enhance leveling. Cotton fabric (51 g) was used with 0.5 g/L of FeSO₄ following the JFL procedure outlined in the experimental section. Upon completion of the mordanting step, the fabric was rinsed in the JFL for 1 min then the dye solution was added. After drying, the fabric was unlevel and had brown speckles. The JFL experiment was repeated (50 g cotton fabric and 0.5 g/L of FeSO₄) for the mordanting process only. Upon completion of the mordanting step the fabric was rinsed for 1 min, removed from the JFL, dyed in the Ahiba Nuance, and dried in a 40°C electric oven. The fabric was still unlevel, had speckles, and migration continued to be a problem.
Figure 18. Photograph of untreated cotton and cotton mordanted with FeSO₄ and dyed with aqueous extracted solution.

Figure 19. Photograph of untreated cotton and cotton mordanted with FeSO₄ and dyed with IPA extracted solution.

4.2.2. Aluminum Sulfate

The experimental process with Al₂(SO₄)₃ followed much of the same path as that of FeSO₄. A liquor ratio of 40:1 with 80% (owf) of Al₂(SO₄)₃ was used as the starting point.
The application procedure for the Ahiba Nuance was used and the fabric samples were transferred to the dye bath directly from the mordant bath. Upon examination of the mordant bath and the fabric, the percent (owf) Al$_2$(SO$_4$)$_3$ was reduced to 20% and the liquor ratio changed to 100:1. The method for the Ahiba Nuance was used again but once the cotton fabric was removed from the mordant bath it was rinsed in cool tap water and run through the padder to remove excess liquid. The percent mordant and liquor ratio were regarded as too high to feasibly be scaled up for commercial use.

In a subsequent set of experiments, Al$_2$(SO$_4$)$_3$ was applied at 3% (owf) with a wet pick up of 100% and the fabric samples were used in the dyeing step. The percent (owf) of Al$_2$(SO$_4$)$_3$ with this method was better based on visual examination, and this experiment was repeated but after padding the cotton was dried in a 45°C mechanical convection oven then used in the dyeing stage. The Al$_2$(SO$_4$)$_3$ level still seemed high so it was reduced to 1% (owf) and wet pickup was 150%.

Using the information gained from the padding experiments, 10% (owf) Al$_2$(SO$_4$)$_3$ was used and the liquor ratio was decreased to 20:1 and mordant was applied using the Mathis Labomat. Fabric was removed from the mordant bath and dried in a mechanical convection oven at 50°C. It was still difficult to achieve level dyeings at this stage (Figure 20).

The JFL was also used to apply the Al$_2$(SO$_4$)$_3$ mordant. In these experiments, cotton fabric (57.94 g) was used with 0.5 g/L of Al$_2$(SO$_4$)$_3$ following the JFL procedure outlined in the experimental section. The fabric was rinsed in the JFL for 1 min then dyed. The
resultant fabric was unlevel, had speckles, and migration occurred during the drying process (Figure 21).

Figure 20. Photograph of untreated cotton, cotton mordanted with Al$_2$(SO$_4$)$_3$ and dyed with IPA extracted solution, and cotton mordanted with Al$_2$(SO$_4$)$_3$ and dyed with the aqueous extract.

Figure 21. Photograph of untreated cotton, cotton mordanted with Al$_2$(SO$_4$)$_3$ in JFL, and cotton mordanted with Al$_2$(SO$_4$)$_3$ and dyed with the aqueous extract in the JFL.
4.2.3. Tannic Acid and Aluminum Sulfate

Using a multi-step mordanting procedure employing tannic acid followed by Al$_2$(SO$_4$)$_3$ and soda ash then a sodium phosphate fixative treatment after dyeing yielded the best results. Cotton fabric samples were level and had no speckles (Figure 22). Also, good washfastness, and lightfastness were achieved. Table 12 summarizes the tannic acid and Al$_2$(SO$_4$)$_3$ mordant and dyeing studies.

Table 12. Summary of conditions used in tannic acid and Al$_2$(SO$_4$)$_3$ mordant and dyeing studies.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Tannic acid Bath</th>
<th>Al$_2$(SO$_4$)$_3$ and Soda ash Bath</th>
<th>Dyeing Cycle</th>
</tr>
</thead>
</table>
| Trial 1 | • Bath removed from heat   
          • In bath for 24-h | • 24-h treatment   
          • Dried at 35°C | 24 h          |
| Trial 2 | • Bath maintained at 70 ±10°C   
          • In bath for 30-min | • 30-min treatment   
          • Dried on conveyor oven | 24 h          |
| Trial 3 | • Bath removed from heat   
          • In bath for 2-h | • 24-h treatment   
          • Dried at 35°C | 24 h          |
| Trial 4 | • Bath maintained at 70 ±10°C   
          • In bath for 2-h | • 2-h treatment   
          • Dried at 35°C | 24 h          |
| Trial 5 | • Bath removed from heat   
          • In bath for 2-h | • 2-h treatment   
          • Dried at 35°C | 24 h          |
| Trial 6 | • Bath removed from heat   
          • In bath for 2-h | • 2-h treatment   
          • Dried at 35°C | Until cooled to 25°C 3 h |
4.3. Dyeing Studies

The tobacco dust extracts from bench-top extractions were used for the dyeing process initially. The solvent used for extraction slightly changed the color of the resulting dyed cotton using both mordants. Dyeings conducted using 2% (owf) tobacco dust extract were very light in color. Once the concentration of tobacco dust extract was increased to 20% (owf) a better color was achieved although the color was still light.

4.3.1. K/S and L*a*b* Data

K/S values were collected at 350 nm wavelength, using samples 1-6. Sample 1 was obtained from dye trial 1, the long mordanting and dyeing procedure. Sample 2 was obtained from mordant trial 2, shortened procedure. Sample 3 was obtained from mordant trial 3 where heat was turned off once the bath reached the desired temperature range was reached, and the tannic acid and fabric were added. The bath was then allowed to cool to room
temperature prior to removing fabric samples (2 h). Sample 4 was obtained from mordant trial 4 (Short B) in which the tannic acid bath containing the fabric sample was held at the target temperature for 2 h then fabric was removed. Sample 5 was obtained from mordant trial 5, the same tannic acid mordant procedure as Sample 3, but the fabric stayed in the Al$_2$(SO$_4$)$_3$ bath for 2 h and the dyed fabric was allowed to remain in the dye bath over night. Sample 6 was obtained from mordant trial 6, the same procedure as Sample 5 except the fabric was removed from the dye bath once the temperature of the bath cooled to 25°C.

Figure 23. K/S values resulting from different tannic acid-Al$_2$(SO$_4$)$_3$ mordanting and dyeing procedures.

Figure 23 shows the K/S values for fabric samples containing only mordant and the corresponding samples that contain mordant and dye. The samples mordanted and dyed using the procedure in Trial 1 gave the highest K/S value, while Trial 2 samples, which
underwent the shortest mordanting and dyeing procedure gave the lowest K/S value.

Allowing the tannic acid mordant bath to cool to room temperature over 2 h (Trial 3) gave a higher K/S value than if the temperature of the tannic acid mordant bath was held elevated for 2 h. Sample 5 showed more improvement in K/S indicating that allowing the fabric samples to sit in the Al₃(SO₄)₃ mordant bath for 2 hr rather than 30 min resulted in a higher K/S value. Sample 6 showed a slightly decreased K/S value due to removal of the fabric sample from the dye bath as soon as it was cooled to 25°C, instead of continuing to sit in the bath overnight.

The dyed samples were evaluated for their L*, a*, and b* values (Table 13). The L* values were consistent with the K/S values. Trial 1 (visually the darkest fabric sample) had the lowest L* value. Trial 6, while it gave a high value for L*, provided a way to accomplish

<table>
<thead>
<tr>
<th>Name</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean Blank Sample</td>
<td>95.33</td>
<td>-0.05</td>
<td>4.96</td>
</tr>
<tr>
<td>Mordant Trial 1</td>
<td>66.13</td>
<td>4.87</td>
<td>23.12</td>
</tr>
<tr>
<td>Mordant Trial 2</td>
<td>75.93</td>
<td>3.81</td>
<td>19.49</td>
</tr>
<tr>
<td>Mordant Trial 3</td>
<td>69.80</td>
<td>5.48</td>
<td>24.94</td>
</tr>
<tr>
<td>Mordant Trial 4</td>
<td>72.96</td>
<td>4.26</td>
<td>21.2</td>
</tr>
<tr>
<td>Mordant Trial 5</td>
<td>68.65</td>
<td>5.26</td>
<td>25.01</td>
</tr>
<tr>
<td>Mordant Trial 6</td>
<td>72.99</td>
<td>4.61</td>
<td>24.05</td>
</tr>
</tbody>
</table>

the entire mordant/dye/fixative process in a single day. This was considerably more practical than Trial 1, which required a week to mordant, dye, and apply the fixative to obtain a finished fabric. Trial 2 was the most accelerated method and while it was also completed in a
single day, the L* value was the lowest of all the trials. Trial 6 provided a good compromise of an acceptable finished product in a reasonable processing time.

4.3.2. Colorfastness to Laundering

Colorfastness to laundering was determined according to AATCC Test Method 61-2004. The change in color was quantified using the AATCC Gray Scale for Color Change. Ratings were assigned to each sample using a scale of 1 (poor) – 5 (excellent). Fastness testing and color change evaluation was repeated on a second set of samples and achieved similar results. A spectrophotometer was also used to evaluate the samples for L*, a*, and b* values. These results are shown in Table 14.

Table 14. L*a*b* values before and after fastness testing of samples resulting from different mordant-dye studies.

<table>
<thead>
<tr>
<th>Name</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>GS Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mordant Trial 1</td>
<td>66.13</td>
<td>4.87</td>
<td>23.12</td>
<td></td>
</tr>
<tr>
<td>Mordant Trial 1 - washfast tested</td>
<td>64.89</td>
<td>5.59</td>
<td>27.88</td>
<td>4-5</td>
</tr>
<tr>
<td>Mordant Trial 2</td>
<td>75.93</td>
<td>3.81</td>
<td>19.49</td>
<td></td>
</tr>
<tr>
<td>Mordant Trial 2 - washfast tested</td>
<td>78.83</td>
<td>3.09</td>
<td>18.57</td>
<td>3-4</td>
</tr>
<tr>
<td>Mordant Trial 3</td>
<td>69.80</td>
<td>5.48</td>
<td>24.94</td>
<td></td>
</tr>
<tr>
<td>Mordant Trial 3 - washfast tested</td>
<td>73.24</td>
<td>4.60</td>
<td>25.31</td>
<td>3</td>
</tr>
<tr>
<td>Mordant Trial 4</td>
<td>72.96</td>
<td>4.26</td>
<td>21.2</td>
<td></td>
</tr>
<tr>
<td>Mordant Trial 4 - washfast tested</td>
<td>77.27</td>
<td>3.42</td>
<td>20.31</td>
<td>3-4</td>
</tr>
<tr>
<td>Mordant Trial 5</td>
<td>68.65</td>
<td>5.26</td>
<td>25.01</td>
<td></td>
</tr>
<tr>
<td>Mordant Trial 5 - washfast tested</td>
<td>70.39</td>
<td>4.78</td>
<td>26.69</td>
<td>4</td>
</tr>
<tr>
<td>Mordant Trial 6</td>
<td>72.99</td>
<td>4.61</td>
<td>24.05</td>
<td></td>
</tr>
<tr>
<td>Mordant Trial 6 - washfast tested</td>
<td>75.60</td>
<td>3.41</td>
<td>22.06</td>
<td>3-4</td>
</tr>
</tbody>
</table>
Figure 24. K/S values for mordanted + dyed cotton and samples before and after laundering, where samples 1-6 arise from different tannic acid and Al₂(SO₄)₃ mordanting and dyeing methods.

The gray scale ratings indicated medium to good washfastness. As expected, the K/S values (Figure 24) generally decreased after laundering and the L* values increased, except in the case of Trial 1. In Trial 1 the fabric was exposed to both mordant baths for a much longer period of time (24 h) than in any other trials (30 min to 2 h). Metal salt mordants work by bonding natural dye molecules to cellulose and keeping them there (4). Exposing the fabric to the mordant bath for a longer period of time could have allowed more mordant to adhere to the fabric, which could then hold more dye to cellulose. This explains why the Trial 1 sample was the visually darkest of the pre-wash tested samples. Next, looking at the a* values, Trial 1 was the only case in which the a* value increased, this causing a more reddish shift. The b* value increased causing a shift less bluish. These shifts could have
changed the cast of the dyed fabric so that it would visually look a little darker and also caused the K/S and L* values to look as if the fabric became darker after wash testing.

4.3.3. Colorfastness to Light

Colorfastness to light was determined according to AATCC Test Method 16-2004, on duplicate fabric samples from each of the mordanting trials. Ratings were assigned to each sample using a scale of 1 (poor) – 5 (excellent). Fastness testing and color change evaluation was repeated on a second set of samples with consistent results and the mean of these values was recorded. A spectrophotometer was also used to evaluate the samples for K/S (Figure 25) and L*, a*, and b* values (Table 15).

<table>
<thead>
<tr>
<th>Sample</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Color Change Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mordant Trial 1</td>
<td>66.13</td>
<td>4.87</td>
<td>23.12</td>
<td></td>
</tr>
<tr>
<td>Mordant Trial 1 - lightfast test (mean)</td>
<td>69.05</td>
<td>3.79</td>
<td>25.79</td>
<td>4</td>
</tr>
<tr>
<td>Mordant Trial 2</td>
<td>75.93</td>
<td>3.81</td>
<td>19.49</td>
<td></td>
</tr>
<tr>
<td>Mordant Trial 2 - lightfast test (mean)</td>
<td>80.37</td>
<td>2.32</td>
<td>18.07</td>
<td>4</td>
</tr>
<tr>
<td>Mordant Trial 6</td>
<td>72.99</td>
<td>4.61</td>
<td>24.05</td>
<td></td>
</tr>
<tr>
<td>Mordant Trial 6 - lightfast test (mean)</td>
<td>76.91</td>
<td>2.88</td>
<td>24.03</td>
<td>4</td>
</tr>
</tbody>
</table>
Gray scale rating indicated good lightfastness. According to the K/S values (Figure 25) it appeared that the fabric from Trial 1 became darker after exposure to light. However, upon examination of the L*a*b* values (Table 15) it was seen that other shifts were taking place. The L* value increased after exposure to light indicating the color faded. Examination of the b* values showed a better indication of the shift that took place. For Trial 1, the fabric became less bluish but in the fabrics from the other trials the b* value decreased (more bluish) or stayed very close to the same. This shift in bluishness could be just enough to change the cast of the dye ever so slightly indicating that fabric samples treated according to Trial 1 faded such that they became less bluish than fabric samples treated according to the other methods. This would cause a change in the K/S value.
Once mordant trial 6 was chosen as the optimum method for mordanting, dyeing, and application of fixative, it was applied using extracts from the three different samples of tobacco (T-1, T-2, and T-3), to determine if the tobacco sample produced differing effects when applied to cotton. The gray scale ratings for colorfastness to light are in Table 16. The K/S values (Figure 26) and L*a*b* values (Table 16) were obtained using a spectrophotometer.

![Figure 26](image)

**Figure 26.** K/S values for mordanted and dyed fabrics before and after laundering and light exposure using the optimized mordanting and dyeing method with the three tobacco dust varieties (T-1, T-2, and T-3).
Table 16. Gray scale ratings and L*a*b* values, for mordanted and dyed fabrics before and after laundering, and light exposure using the optimized mordanting and dyeing method with the three tobacco dust samples (T-1, T-2, and T-3).

<table>
<thead>
<tr>
<th>Sample</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Color Change Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-1</td>
<td>72.99</td>
<td>4.61</td>
<td>24.05</td>
<td></td>
</tr>
<tr>
<td>T-1 washfast tested</td>
<td>75.60</td>
<td>3.41</td>
<td>22.06</td>
<td>3-4</td>
</tr>
<tr>
<td>T-1 mean lightfast tested</td>
<td>76.91</td>
<td>2.88</td>
<td>24.03</td>
<td>4</td>
</tr>
<tr>
<td>T2</td>
<td>70.38</td>
<td>5.07</td>
<td>23.60</td>
<td></td>
</tr>
<tr>
<td>T2 washfast tested</td>
<td>74.13</td>
<td>3.98</td>
<td>21.99</td>
<td>3-4</td>
</tr>
<tr>
<td>T-2 mean lightfast tested</td>
<td>73.83</td>
<td>4.41</td>
<td>23.06</td>
<td>4</td>
</tr>
<tr>
<td>T-3</td>
<td>71.75</td>
<td>4.97</td>
<td>22.83</td>
<td></td>
</tr>
<tr>
<td>T-3 washfast tested</td>
<td>72.36</td>
<td>5.26</td>
<td>23.88</td>
<td>3-4</td>
</tr>
<tr>
<td>T-3 mean lightfast</td>
<td>72.01</td>
<td>5.04</td>
<td>28.73</td>
<td>4</td>
</tr>
</tbody>
</table>

Visually the shades for the mordanted and dyed fabric were similar (Figure 26 on previous page). The shades for fabrics dyed with T-1 and T-2 extracts and washed were very similar and type T-3 was a bit darker. T-3 was the darkest colored tobacco dust with T-2 the lightest and T-1 in the middle. As expected for T-1 and T-2 dyed fabrics, the K/S value decreased after laundering and light exposures. The K/S values from T-3 appeared to increase after laundering and light exposure. Upon examination of the L*a*b* values (Table 16), however, the L* values from all three increased after laundering and light exposure. Using T-3, the increased a* value again showed a shift in the opposite direction of the other two varieties where the a* value decreased. This indicated that when washed the fabric from T-3 lost some of its greenness, becoming slightly more red. This may account for the change in K/S value. Fabric samples from T-3 that underwent light exposures had an increased b*
value, indicating that T-3 became less bluish. This shift caused a more reddish cast to the fabric.

4.3.4. Crockfastness

Fastness to dry crocking was evaluated using the AATCC Test Method 8-2000. The change in color was assigned using the AATCC Chromatic Transference Scale. A rating of 1 (poor) – 5 (excellent) was given to each fabric sample. Samples from Trials 1-6 using the combined tannic acid and Al$_2$(SO$_4$)$_3$ mordanting method, dyed with 1% dye solution and treated with the sodium phosphate fixative had a fastness rating of 5 (Table 17). Samples using the optimized procedure for mordant, dye, and fixative using dye stock solutions made from each of the three tobacco dust samples also had a rating of 5 (Table 17).

The combination of the tannic acid mordant treatment with the Al$_2$(SO$_4$)$_3$ mordant, then the sodium phosphate fixative produced dyed fabrics that had a high crock fastness rating. This indicated penetration of dye into the fiber.

Table 17. Gray scale ratings for crockfastness using samples from tobacco dust T-1, T-2, and T-3 extracts.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Color Change Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mordant Trial 1</td>
<td>5</td>
</tr>
<tr>
<td>Mordant Trial 2</td>
<td>5</td>
</tr>
<tr>
<td>Mordant Trial 3</td>
<td>5</td>
</tr>
<tr>
<td>Mordant Trial 4</td>
<td>5</td>
</tr>
<tr>
<td>Mordant Trial 5</td>
<td>5</td>
</tr>
<tr>
<td>Mordant Trial 6</td>
<td>5</td>
</tr>
<tr>
<td>Optimized T-1 Trial</td>
<td>5</td>
</tr>
<tr>
<td>Optimized T-2 Trial</td>
<td>5</td>
</tr>
<tr>
<td>Optimized T-3 Trial</td>
<td>5</td>
</tr>
</tbody>
</table>
4.4 UV-VIS

UV-VIS analysis was performed on extracts of T-3 obtained using tap water (Figure 27). UV-VIS spectroscopy showed peaks at 254 and 259 nm and the UV_VIS spectrum for the sample obtained using IPA (Figure 28) yielded peaks at 607, 667, 532, and 401 nm. However, these peaks are very small.

Figure 27. UV-VIS spectrum of extract from tobacco dust T-3 using bench-top H₂O extraction (120-min) method.
Figure 28. UV-VIS spectrum for IPA extract from tobacco dust T-3.

Spectra from aqueous and IPA extracts lacked strong peaks in the visible region. There was a peak with a tail in the visible region, indicating an absorbance of violet light (380 to 420 nm) and an observed yellow color, but that the colorants in the extract would be a weak yellow compound.

4.4.1 Determination of Dye in Solution

Linear regression of the calibration data was used to generate a calibration curve (Figure 29). The measured absorbances of the residual dyebaths were used with the Beer-Lambert Law or Beer’s Law to generate regression models to determine the concentration of dye in each solution. $R^2$ for the calibration curve at 380 nm was 0.99989. The calibration
equation was: \[ \text{Absorbance} = 0.73193 \times \text{concentration}. \]

\[ Absorbance = 0.73193 \times \text{concentration} \]

![Graph showing Calibration Curve at 380 nm for determination of dye in dye baths pre- and post-dyeing cycle.]

Figure 29. Calibration Curve at 380 nm for determination of dye in dye baths pre- and post-dyeing cycle.

Stock solutions and post-dyeing bath solutions required dilution for measurements in the appropriate absorbance range by the UV-VIS spectrophotometer. The extracts of T-1, T-2, and T-3, were used to make a 1% stock solution, and the solutions were diluted 1:10 with de-ionized water. The post dye path solutions of T-1, T-2 and T-3 were all diluted 1:1 with de-ionized water and absorbance was measured at 380 nm. Concentrations and absorbances before and after dyeing with the three different tobacco dust varieties can be seen in Table 18. Absorbance increased after completion of a dye cycle. This corresponds with the change in concentration of dye in the dye bath pre- and post dye cycle. The T-1 dye bath went from
a 10-mg/mL concentration to 3.8 mg/mL with only one dye cycle this was a 61.6% 
exhaustion of colorant. The T-2 and T-3 dye baths experienced 77% exhaustion.

Table 18. Pre- and post dyeing concentration and absorbances determined from UV-VIS 
analysis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution Factor</th>
<th>Diluted Concentration mg/mL</th>
<th>Actual Concentration mg/mL</th>
<th>Absorbance (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-1 Pre-Dye Bath</td>
<td>10</td>
<td>0.986</td>
<td>9.9</td>
<td>0.722</td>
</tr>
<tr>
<td>T-2 Pre-Dye Bath</td>
<td>10</td>
<td>1.063</td>
<td>10.6</td>
<td>0.778</td>
</tr>
<tr>
<td>T-3 Pre-Dye Bath</td>
<td>10</td>
<td>1.267</td>
<td>12.7</td>
<td>0.928</td>
</tr>
<tr>
<td>T-1 Post Dye Bath</td>
<td>2</td>
<td>1.905</td>
<td>3.8</td>
<td>1.394</td>
</tr>
<tr>
<td>T-2 Post Dye Bath</td>
<td>2</td>
<td>1.193</td>
<td>2.4</td>
<td>0.873</td>
</tr>
<tr>
<td>T-3 Post Dye Bath</td>
<td>2</td>
<td>1.482</td>
<td>2.9</td>
<td>1.085</td>
</tr>
</tbody>
</table>

4.5 High Performance Liquid Chromatography

HPLC was performed on extracts of tobacco dust T-3. The first chromatogram 
(Figure 30) and spectra (Figure 31 and Figure 32) were from extracts using H₂O as the 
solvent. The chromatogram of the H₂O extract of T-3 at 351 nm was typical of the H₂O 
extracts. There was poor separation between the peaks that eluted quickly and peaks 
occurring later were not sharp.
Figure 30. Chromatogram of T-3 aqueous extract at 380-nm.
Figure 31. Absorption spectrum recorded on LC of T-3 aqueous extract eluting at 0.658 min.
Figure 32. Absorption spectrum recorded on component of T-3 aqueous extract eluting in LC at 5.328 min.
Figure 33. Chromatogram of IPA extract of T-3 tobacco dust.

Figure 34. Absorption spectrum recorded on component of T-3 IPA extract eluting in LC at 0.594 min.
Figure 35. Absorption spectrum recorded on component of T-3 IPA extract eluting in LC at 12.237 min.

The chromatogram of the IPA extract of T-3 tobacco dust (Figure 33) was typical of chromatograms for tobacco dust extracted with IPA. There was poor separation of peaks, but the peaks were larger than those from the aqueous extract. The spectra recorded on components of T-3 IPA extract showed (Figure 34 and Figure 35) very weak peaks in the visible region, appearing as low intensity tails.
4.6. Nicotine Analysis

LC-MS was used to determine semi-quantitatively the amount of nicotine in the tobacco dust extract, whether it transferred to the fabric when dyeing with the tobacco dust extract, and whether it was washed out during laundering associated with the AATCC washfastness to laundering test method. The same method and column were used for LC-MS nicotine analysis as was used for colorant analysis.

Figure 36. Extracted ion chromatogram showing the peak for nicotine.

Figure 37. ESI Mass spectrum for nicotine.
Upon confirmation that nicotine was in the tobacco dust extract and in the dyed cotton fabric, a calibration curve was used to determine quantitatively the percent of nicotine present T-1 and cotton fabric. Standards were made from a 10.16-mg/10 mL stock solution of nicotine and HPLC grade de-ionized water. The calibration curve obtained had an $R^2$ value of 0.99434 (Figure 38). The equation for the slope of the calibration curve was:

$$y = 291846 \times x$$

While this is not a very high an $R^2$ value, it does give a general idea of how much nicotine was in the extract, the dyed fabric, and the laundered fabric. One reason the $R^2$ value was a little low is that the trend line was forced through the origin so as to have a $y$-intercept of 0.

Figure 38. Calibration curve for nicotine in H$_2$O.
The area of the LC-MS peak for nicotine (163.12340) from the T-1 extract was 11,688,677. This value was used as the y-value in the slope equation. Solving for x, gave the nicotine content in ppm for both the tobacco dust extract and the cotton fabric. The mass of the cotton fabric used for nicotine extraction studies was 1450 mg. The volume of T-1 extract was 138 mL (1:100 dilution). This enabled the determination that the extracted T-1 tobacco dust extract contained 3.1% nicotine. The dyed cotton contained 0.03 % nicotine (owf). The washed fabric contained 0.0006% nicotine (owf).

It is known the amount of nicotine inhaled from 1 cigarette is between 1 and 2 mg and the lethal dose for an adult is 30 to 60 mg (97, 98). A women’s small 100% cotton t-shirt weighs around 116 g. If this t-shirt had been dyed with the T-1 extract, at the 0.03% owf nicotine level the t-shirt would contain 35.74 mg of nicotine. While it is unlikely that one would absorb all the nicotine in the t-shirt through the skin all at once, this level is in the range of a lethal dose of nicotine. If this t-shirt had been washed according to the AATCC test method for colorfastness to laundering then it would contain 0.66 mg of nicotine, slightly less than is absorbed by the body by smoking a single cigarette.

4.7. Colorant Characterization

LC/MS was performed on the fractions obtained from dry column chromatography of T-1 aqueous extract, to help determine the colorants present. The developed column (Figure 39) showed multiple colored bands, with appreciable color remaining at the top of the column. Use of 1:1 ethyl acetate: methanol as the eluent indicated that while one component
was relatively non-polar and raced down the column very quickly (fraction 4), much of the extract was quite polar.

Figure 39. Low-pressure chromatography column of T-1 tobacco extract.

Thin-layer chromatography (TLC) was performed on fractions 1, 2, 3, and 4 with rutin and fustic used as standards. A previous report indicated rutin, quercetin, and apigenin were components of tobacco dust extracts (60). TLC analysis suggested that rutin was present and that fustic was not a likely component in the T-1 extract.

The fractions were then subjected to LC/MS analysis looking for rutin, quercetin, and apigenin using the same method developed for nicotine (76). Rutin was prepared as a standard. The UV-VIS spectrum for rutin is shown in Figure 40. The peak centered at 360 nm in the ultra-violet region extends above 380 nm. This was consistent with the UV-VIS spectrum obtained on samples of tobacco dust extract using IPA and water. The mass spectrum of rutin is shown in Figure 41. Rutin has a mass to charge ratio of 611.101 m/z and elutes at 4.339 min.
Figure 40. UV-VIs spectrum for rutin.

Figure 41. ESI Mass spectrum for rutin.

Fraction 1 results are shown in Figure 42. When the extracted ion chromatogram for rutin was examined at 380 nm there was a peak near the 4.34-min mark. This indicated that rutin was a component in fraction 1 of the T-1 aqueous extract. However, it appeared to be
Figure 42. LC/MS chromatograms for fraction 1. Top: DAD for fraction 1.  2nd: Extracted ion chromatogram for rutin.  3rd: Extracted ion chromatogram for apigenin.  Bottom: Extracted ion chromatogram for quercetin.

in smaller quantities than the literature had suggested (60). Analysis for quercetin (bottom) did not show this compound to be a component in the T-1 extract. The same was the case for apigenin, as seen in the third extracted ion chromatogram.

Fraction 2 was examined for quercetin, rutin, and apigenin (see Figure 43). The extracted ion chromatogram did not contain clear peaks around the 4.334 min mark where rutin elutes. This indicated that it was either not present in fraction 2 or it was present in such small amounts that it could not be detected. The same was true for the quercetin (top chromatogram) and apigenin (bottom chromatogram).
Figure 43. Extracted ion chromatograms for fraction 2. Top: quercetin. Middle: rutin. Bottom: Apigenin.

The third (Figure 44) and fourth (Figure 45) fractions were examined for rutin, quercetin, and apigenin. The results were consistent with those from fraction 2. The peaks were examined by mass spectrum and showed no indication of rutin, quercetin, or apigenin.
Figure 44. Extracted ion chromatograms for fraction 3. Top: quercetin, Middle: rutin, Bottom: apigenin.

Figure 45. Extracted ion chromatograms for fraction 4. Top: quercetin, Middle: rutin, Bottom: apigenin.
Since this analysis was not in agreement with analysis performed by others, the previous method was examined to determine whether it could be reproduced (60). Published details were very vague, as no temperature was specified, nor length of time for “percolation.” Percolation was not defined either. This rendered it impossible to fully reproduce the extraction process used. Instead, a T-1 tobacco dust was packed into a 1-in diameter nylon tube used for low-pressure chromatography along with glass wool and sand to prevent the tobacco dust from falling out of the tube and solvent was allowed to drip through the tobacco dust and out the bottom of the tubing to be collected. Methanol (70%) and water (30%) were used (100 ml) as the solvent and allowed to slowing drip through the tobacco dust. The solvent was collected until it was colorless. The methanol was evaporated using a rotary evaporator. Then LC/MS was used to determine if rutin, quercetin, and/or kaempferol were present in this extract. The results are shown below in Figure 46. The top chromatogram contains the peaks extracted from the 70% MeOH extracted sample. The second chromatogram shows the chromatogram extracted to find kaempferol. There was no clear peak. The same was the case for the third chromatogram extracted looking for quercetin. The fourth chromatogram gave a clear peak indicating the presence of rutin, but it did not seem to be as large a peak as the previous reports indicated (60).
Figure 46. Chromatograms for 70% methanol extract of T-1 tobacco dust. Top: Total ion chromatogram, 2nd: extracted ion chromatogram for kaempferol, 3rd: quercetin, Bottom: extracted ion chromatogram for rutin.

This analysis indicated that while rutin was present, quercetin and apigenin were not present in the extracts obtained in this work. Since others have found the presence of these compounds there are a couple possibilities to explain this discrepancy. It is possible that the tobacco dust used in the previous studies was percolated for many hours, days, or even weeks. An unknown mixture of fermented, unfermented, and waste tobacco leaves were used whereas the present work used a homogeneous tobacco dust. The quantity of rutin and other compounds is different at different stages of the curing process. For instance, flue-cured tobacco contains a higher concentration of rutin than air-cured types (103–105). Due to the mixture of tobacco at different stages in the process it would be logical for the results of constituent compounds vary. Another explanation is that they extraction process used for
the T-1 tobacco dust was too aggressive. The use of the pressure in the ASE and the high temperature (120°C) may cause unintended changes in the constituents.

4.8. Atomic Absorption

Atomic absorption analysis was used to determine the percent Fe absorbed by the cotton knit fabric under different treatment conditions (Table 19). Two samples of cotton treated only with the mordant bath, one sample cotton treated with the mordant bath followed by dyeing, and two samples of untreated cotton were used in this experiment. Standards of 200, 400, and 600-µL Fe standard (PerkinElmer Pure Iron 2% HNO₃ Lot # 17-49Fe) at a 1:1 ratio, diluted to 100 mL and used to create the calibration curve with the corresponding $R^2$ of 1.0000 (Figure 47).

![Figure 47. Calibration curve for the atomic absorption of Fe with $R^2 = 1.0000$.](image-url)
Table 19. Percent of Fe found on fabric samples (clean/untreated, mordanted, and mordanted/dyed) by Atomic Absorption.

<table>
<thead>
<tr>
<th>Fabric</th>
<th>Mass of Fabric Digested (g)</th>
<th>Fe Found (ppm)</th>
<th>% Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean/Untreated 1</td>
<td>0.23421</td>
<td>0.210</td>
<td>0.009%</td>
</tr>
<tr>
<td>Clean/Untreated 2</td>
<td>0.25270</td>
<td>0.281</td>
<td>0.011%</td>
</tr>
<tr>
<td>Mordant Only 1</td>
<td>0.25745</td>
<td>3.229</td>
<td>0.125%</td>
</tr>
<tr>
<td>Mordant Only 2</td>
<td>0.25094</td>
<td>2.527</td>
<td>0.101%</td>
</tr>
<tr>
<td>Mordant w/ Dye</td>
<td>0.24023</td>
<td>1.691</td>
<td>0.070%</td>
</tr>
</tbody>
</table>

The untreated cotton contained a mean value of 0.010 % Fe, mordanted and dyed cotton contained 0.070 % Fe, and mordanted only cotton contained a mean value of 0.113 % Fe. The trace amount found in the untreated cotton most likely results from contact with water containing trace amounts of Fe in the wet processing of the cotton prior to its use in this project. It seems logical that there is less Fe on the fabric that has been mordanted and dyed. Some of the Fe was likely washed out in the dye bath. This would explain why the mordant only treated cotton contained more Fe than the cotton that had been mordanted and dyed.

**Chapter 5. Conclusion**

Tobacco dust generated as a waste product of cigarette manufacturing has been shown to contain constituents suitable for coloring cotton fibers. Use of an Accelerated Solvent Extractor provided an efficient and easy way to remove these components from commercial tobacco dust, using water or an organic solvent as the medium. With the aid of this technique, it was possible to generate tobacco extract yields of up to 40% by mass brown material for dyeing studies.
Although Al$_2$(SO$_4$)$_3$ and FeSO$_4$ can be used as mordants in dyeing cotton with tobacco dust extract, Al$_2$(SO$_4$)$_3$ in conjunction with tannic acid proved to be the mordant system of choice. For the dye application step, an Ahiba Nuance or Mathis Labomat machine gave good results (i.e. level dyeings). Analysis of the dye baths showed a mean exhaustion level of 72% for one dye cycle but the total dyeing time was 8 h.

The K/S values from all dyeing studies were modest (<4), with khaki colors consistently achieved. However, washfastness and lightfastness results from a standard 2A washfastness test and a 20-h light exposure were good (GS ratings 3-4).

UV-VIS spectra from aqueous and IPA extracts lacked strong peaks in the visible region. The observed color was due to absorption peaks that tailed into the visible region up to 420 nm. Isolation of these components using column chromatography gave solutions having a weak yellow color.

Colorants and nicotine content in tobacco dust extracts were analyzed using LC-MS. Rutin was the only chromogen confirmed in the extracts obtained although others were present. The nicotine content of the T-1 tobacco dust extract was 3.1% (by weight), while the dyed fabric contained 0.03% nicotine (owf) and the washed dyed fabric contained 0.0006% nicotine (owf).

The results of this study suggest that tobacco dust merits further evaluation as a natural dye source for textile coloration. A better understanding of the colorants present and a way to shorten the required dyeing time are key next steps.
REFERENCES


<table>
<thead>
<tr>
<th>No.</th>
<th>Author(s)</th>
<th>Title and Source</th>
</tr>
</thead>
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