

Analysis of the chemical composition of cotton seed coat by Fourier-transform infrared (FT-IR) microspectroscopy

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Abstract To improve knowledge on the biodegradation of cotton seed coat fragments in bioscouring, in this study Fourier-transform infrared (FT-IR) microspectroscopy was performed to analyze the composition of cotton seed coat. Microscope observation showed that cotton seed coat has a five-layer structure: epidermal layer, outer pigment layer, colorless layer, palisade layer, and inner pigment layer. Analysis of the FT-IR spectra from each layer shows that cutin, wax, cellulose, and pectin are the main components of the epidermal layer, while pectin and hemicellulose are the main constituents of the palisade layer, as well as aromatic and polyphenol, which are commonly considered as lignins. The main component of the outer and inner pigment layers is lignin. The results suggest that cellulase, pectinase, xylanase, and lignin oxidase are suitable for degradation of cotton seed coat. In addition, cutinase might be very promising in improving the enzymatic degradation of cotton seed coat.

Keywords Composition · Cotton seed coat · Fourier-transform infrared (FT-IR) microspectroscopy · Degradation

Introduction

The bioscouring process is an ideal substitute for the alkaline scouring process due to its advantages of mild reaction condition, lower energy consumption, and environmental protection. Cotton seed coat fragments are the most resistant impurities in cotton fabric following bioscouring. The incomplete removal of cotton seed coat fragments during bioscouring cannot satisfy the subsequent dyeing process, which remains an obstacle to the commercialization of the bioscouring process.

Much work has been done to improve the biodegradation of cotton seed coat (CSC). Enzymes such as cellulase, pectinase, and hemicellulase were reported to accelerate the degradation of holocellulose in cotton seed coat fragments, especially when applied together with ethylene diamine tetraacetic acid (EDTA) (Csiszár et al. 1998, 2001). Csiszár et al. (2006) suggested that xylanase pretreatment can destroy the lignocellulosic structure of cotton seed coat with the delignification and the lightness of the substrate enhanced in the subsequent alkaline scouring process. Cellulase can also degrade the lignocellulose in CSC (Csiszár et al. 1998). Recently,

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enzymes extracted from solid fermentation which utilized cotton seed coat fragment waste as the carbon source were used for cotton bioscouring to improve the removal of cotton seed coat fragments from cotton fabric (Csiszár et al. 2007).

It is the solid structure and complex composition of CSC that cause the difficulties in its biodegradation. Few researchers have attempted to study the structure and complex composition of CSC for its effective biodegradation. We believe that good knowledge about the structure and composition of CSC may help to study its biodegradation. However, until recently, information about the composition of CSC was very incomplete. Further studies are still necessary.

The purpose of this paper is to study the composition of CSC. The structure of CSC was investigated based on observation of its cross-section. FT-IR microspectroscopy technology, which has been successfully and widely used to study the composition and localized changes in plant cell walls (Stewart 1996; McCann et al. 1992; Nicholas et al. 2001; Sørensen et al. 2000; Morrison et al. 1999; Stewart et al. 1995; Himmelsbach et al. 1999, 2002), was used to analyze the composition of each layer of the CSC.

Materials and methods

Materials

Cotton seeds used in this work were from Yancheng City, Jiangsu Province, and were harvested in 2006. All the cotton seeds used in this study were randomly selected.

Methods

Section

Fibers were removed from the seeds by forceps before the samples were dehydrated in a series of ethanol-water solutions with increased ethanol concentrations. The samples were sequentially immersed in increasing ethanol concentrations (50%, 70%, and 100% ethanol solutions for 15, 15, and 30 min, respectively). Then the samples were embedded with JB-4 embedding kit and cut into sections with a

thickness of 5–10 μm with LEICA EMUC 6 ultramicrotome for use in light-microscopy observation and FT-IR microspectroscopy analysis (Himmelsbach et al. 2003).

Light microscopy

Sections of CSC were observed under bright-field microscopy by using Nikon Digital Camera DXM 1200C optical microscopy.

FT-IR microspectroscopy

Spectra were obtained on a FT-IR bench integrated with a Nicolet 5700 1200C optical microscope. The system used a globar source and a KBr beam splitter, housed on the bench, and employed a liquid-nitrogen-cooled MCT/A detector, which was located in the microscope. The data were analyzed with Ominic 7.2 software. Each pixel was collected so as to contain an infrared spectrum over a range of 4,000 to 650 cm^{-1} at a spectral resolution of 8 cm^{-1} with 256 scans, physical mirror velocity of 1.8988 cm/s , and bench aperture setting of 1,000. The spectra of the pieces (sections) were background-subtracted (Himmelsbach et al. 2003).

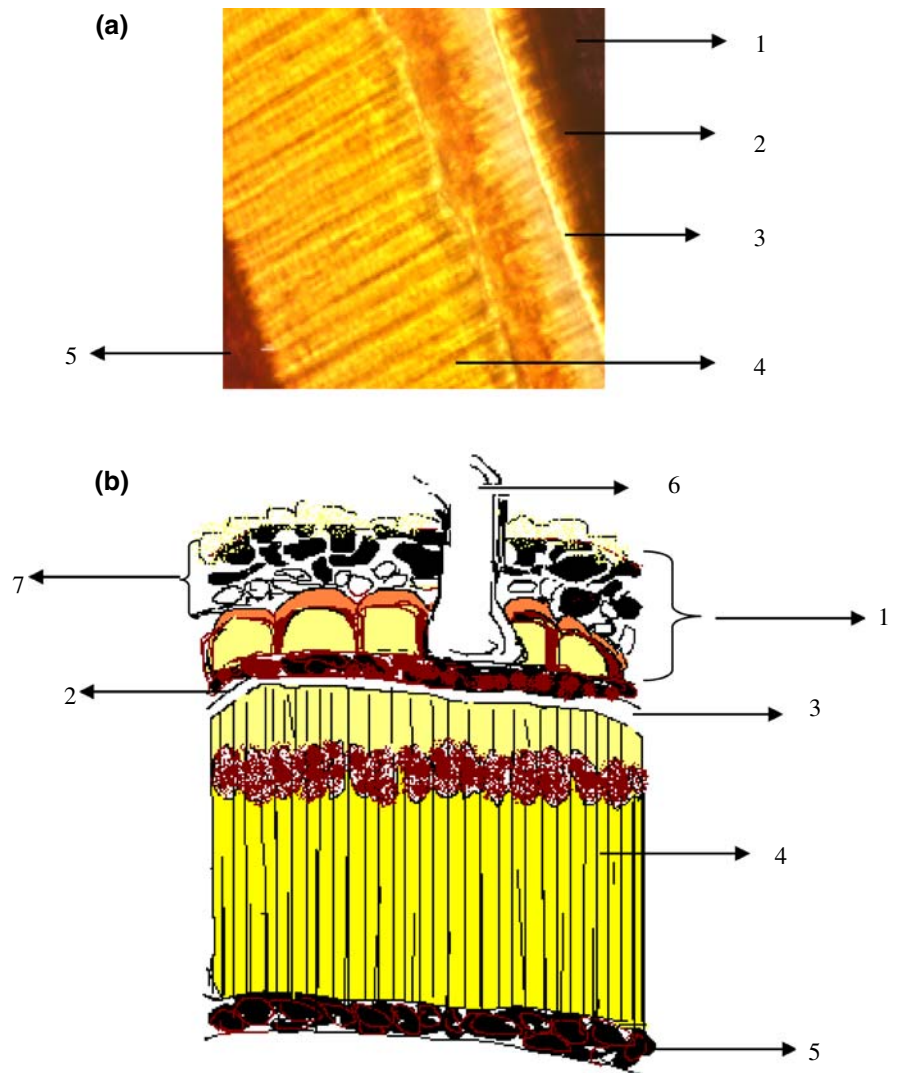
Results and discussion

Structure of cotton seed coat

The CSC cross-section (5–10 μm thick) was observed with bright-field microscopy; a photograph and sketch map of the CSC section are shown in Fig. 1. According to literature reports and the light-microscopy results (Fig. 1), it can be concluded that CSC has a five-layer structure: epidermal layer, outer pigment layer, colorless layer, palisade layer, and inner pigment layer (Himmelsbach et al. 2003; Reeves and Valle 1932).

About 75–85% of the total amount of CSC pollution on cotton fiber is from the palisade layer, which is a single layer of highly elongated cells. The structure of individual palisade cells was found to be rather variable in different regions (Reeves and Valle 1932). The palisade layer differs in thickness and microhardness in chalazal, middle, and micropilar parts of mature cotton seed. On a micropilar end of

Fig. 1 Structure of cotton seed coat cross-section. **a** Bright-field micrograph of the cotton seed coat cross-section; **b** Schematic of a cross-section of cotton seed coat showing anatomical layers. 1 epidermis layer, 2 outer pigment layer, 3 colorless layer, 4 palisade layer, 5 inner pigment layer, 6 cotton fiber, 7 cuticle



cotton seed the palisade layer is twice as thin as in a middle part. In contrast, outer and inner pigment layers in micropilar part are approximately twice as thick as in middle section of cotton seed (Krakhmalev and Sultanova 1983).

Another very important pollution for cotton fiber is from the epidermal layer of some sorts of cotton seed due to reduced durability. The epidermis of CSC is composed of large and irregularly shaped cells. In most aerial plant tissue, there is a protective layer called the cuticle outside the epidermis cell wall. The cuticle has a function as a water-impermeable barrier, which protects plant tissue against water loss and attack by parasites and plant pathogens. Studies on plant epidermis indicate that the cuticle adheres to the

epidermal cell wall by esterified pectin, and that there is no obvious boundary among cutin, pectin, and epidermal cell wall (Doi and Steinbüchel 2002).

The mechanical properties of CSC are responsible for the mechanical resistance of CSC to the removal of seed coat during ginning, consequently leading to the pollution of cotton fiber by CSC fragments. For most mature cotton seeds, the epidermal, outer pigment, and colorless layers have microhardness close to that of annealed aluminum metal, and the palisade layer near the light line has microhardness of annealed copper metal (Krakhmalev and Gershman 1990; Paiziev and Krakhmalev 2006). Measurements of microhardness of separate layers have shown that there is a dramatic jump of microhardness corresponding to the position

of a so-called light line inside the palisade layer. Details about the research into the mechanical properties and microstructure of difference CSC layers can be found in many published studies (Fryxell 1963; Reeves and Valle 1932; Ryser et al. 1988; Krakhmalev and Sultanova 1983; Paiziev and Krakhmalev 2006).

Chemical composition of cotton seed coat epidermal layer

FT-IR spectra of the CSC epidermal layer and the spectra of apple cutin, which was used as a cutin reference, are shown in Fig. 2b. The rectangular box in Fig. 2a delineates the region selected for infrared mapping.

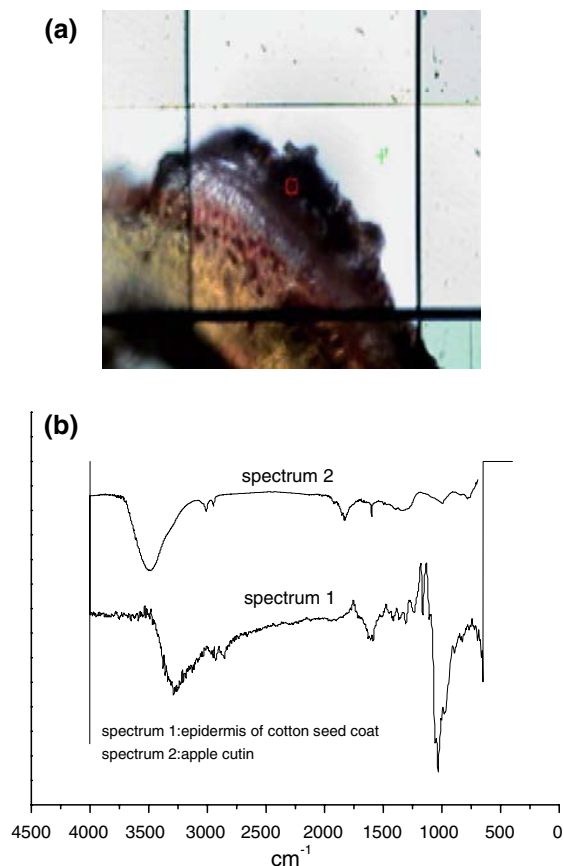


Fig. 2 FT-IR spectra of the epidermis layer of cotton seed coat. **a** infrared mapping; **b** FT-IR spectra of palisade layer of cotton seed coat, *Spectrum 1* epidermis of cotton seed coat; *Spectrum 2* apple cutin (reference sample)

The spectra of CSC epidermal layer and that of apple cutin exhibit similar bands at 2,923 and 2,885 cm^{-1} due to $-\text{CH}_2$ and $-\text{CH}-$ stretch vibration absorbance, respectively (Fig. 2b). These two absorbances normally suggest presence of wax and cutin in plant cell wall, which agrees with the results of Reeves and Valle (1932) and Fryxell (1963). Ryser et al. (1988) and Yatsu et al. (1983) considered that wax in cotton is mainly composed of C18-30 alkyl alcohol, C16-34 alkyl acid, and their esters. This indicates that the potentially diagnostically important bands for wax and cutin are $-\text{CH}_2$ stretch (2,923 cm^{-1}), $-\text{CH}$ stretch (2,885 cm^{-1}), and $\text{C}=\text{O}$ stretch (1,732 cm^{-1}). However, only the bands at 2,923 and 2,885 cm^{-1} are useful for wax and cutin due to the presence of $\text{C}=\text{O}$ in many substance.

The strong set of bands at 1,200 to 1,000 cm^{-1} in spectrum 1 (Fig. 2b) might be ascribed to the absorbance of cellulose (1,161, 1,112, 1,060, 1,031, 982, 898 cm^{-1}) and pectin (1,151, 1,004, 1,082, 1,052, 1,022, 972, 891 cm^{-1}) (Yatsu et al. 1983; Kačuráková et al. 2000). Each polysaccharide has a specific band maximum in the fingerprint region, probably due to the combination of ring vibrations overlapped with side-group ($\text{C}-\text{OH}$) stretch vibrations and glycosidic bond ($\text{C}-\text{O}-\text{C}$) vibration. The absorbance of $\text{C}-\text{OH}$ stretch vibration and $\text{C}-\text{O}-\text{C}$ vibration has a shift of $\pm 10 \text{ cm}^{-1}$ for different polysaccharides due to the effect of the group adjacent to them (Kačuráková et al. 2000; Wilson et al. 2000). Few researchers have reported the existence of pectin in CSC (Himmelsbach et al. 2003; Ryser et al. 1988; Fryxell 1963). Further evidence for the presence of pectin is in the region of 1,780 to 1,200 cm^{-1} in spectrum 1 of Fig. 2b, from which more detailed information about pectin in CSC could be obtained. Absorbances in this region are associated with $\text{C}=\text{O}$ stretch (1,745 cm^{-1}) for pectic acid; $\text{C}=\text{O}$ stretching (1,745 cm^{-1}), $\text{O}-\text{CH}_3$ stretching (1,445 cm^{-1}), and $-\text{CH}_3$ distortion (1,234 cm^{-1}) for pectic ester; and COO^- asymmetric stretch (1,614 cm^{-1}) and symmetric stretch vibration (1,425 cm^{-1}) for calcium pectate (Chung et al. 2004; Wang et al. 2006; Sene et al. 1994). Cellulose is the main component in cotton seed epidermis cell wall (Reeves and Valle 1932). Therefore, cellulase and pectinase are target enzymes for the destruction of cotton seed coat epidermis cell wall. Moreover, cutinase was reported to be able to specifically hydrolyze the cuticle in cotton fiber (Degani et al.

2002; Agrawal et al. 2008), which indicated that cutinase can probably be used for the degradation of aliphatic components in cotton seed coat epidermal layer.

The absorbances in the region of 1,780 to 1,200 cm^{-1} in spectrum 1 (Fig. 2b) indicate that hemicellulose is present in the CSC epidermal layer. C=O stretching (1,732 cm^{-1}), $-\text{CH}_3$ symmetric deformation (1,370 cm^{-1}), and C–C–O asymmetric stretching (1,250 cm^{-1}) are from acetylated glucomannan, which is a hemicellulose component. Unfortunately, those bands are not well enough separated from others to make them very diagnostic because of interferences from other components. Therefore, other methods are needed to confirm the presence of hemicellulose in the epidermal layer of CSC. A band due to bound water is observed in the region between 1,650 and 1,630 cm^{-1} in spectrum 1 in Fig. 2b.

Chemical composition of cotton seed coat palisade layer

FT-IR spectra of palisade layer are shown in Fig. 3b. The rectangular box in Fig. 3a indicates the region selected for infrared mapping. The set of bands in the fingerprint region in Fig. 3b provides some information about the presence of certain polysaccharides in CSC palisade layer. The bands at 1,160, 1,148, 1,108, 1,070, 1,040, 980, 895, and 811 cm^{-1} can be attributed to absorbance by arabinoglucuronoxylan and galactoglucomannan, which are the components of hemicellulose (Kačuráková et al. 2000). The bands at 1,151, 1,004, 1,082, 1,052, 1,022, 972, and 891 cm^{-1} are due to the absorbances of pectin (Kačuráková et al. 2000). The three bands at 1,741, 1,445, and 1,239 cm^{-1} indicate that there is pectin ester in the CSC palisade layer. Further analysis of the presence of pectin acid and its salt was not conducted in this study since C=O absorbance in the range from 1,732 to 1,690 cm^{-1} cannot be well enough separated from other bands to make them very diagnostic. Bands at 1,319 and 1,227 cm^{-1} are C–O stretching vibration for syringyl unit, which indicates that lignin is present in the CSC palisade layer and that syringyl unit is the main lignin type in the CSC palisade layer since there is no evidence of the presence of other lignin types except syringyl unit.

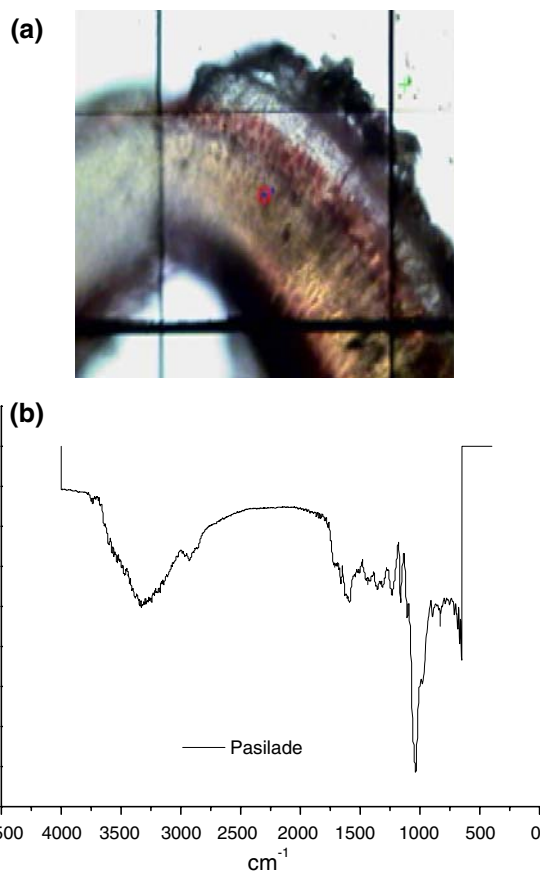


Fig. 3 FT-IR spectra of the palisade layer of cotton seed coat. **a** infrared mapping; **b** FT-IR spectra of palisade layer of cotton seed coat

Most of the CSC fragments on cotton fabric are from the CSC palisade layer. Therefore, the effective removal of cotton seed coat fragments in cotton bioscouring process greatly depends on the degradation of hemicellulose and lignin. Xylanase and lignin peroxidase can be used for their degradation, respectively. Pectin usually acts as cement among the components of plant tissue, therefore the combination of pectinase with xylanase and lignin peroxidase for the degradation of CSC will facilitate the release of enzymatic products.

Chemical composition of cotton seed coat outer and inner pigment layers

CSC outer pigment layer is similar to the inner pigment layer in terms of structure, chemical composition, and developmental course (Ryser et al.

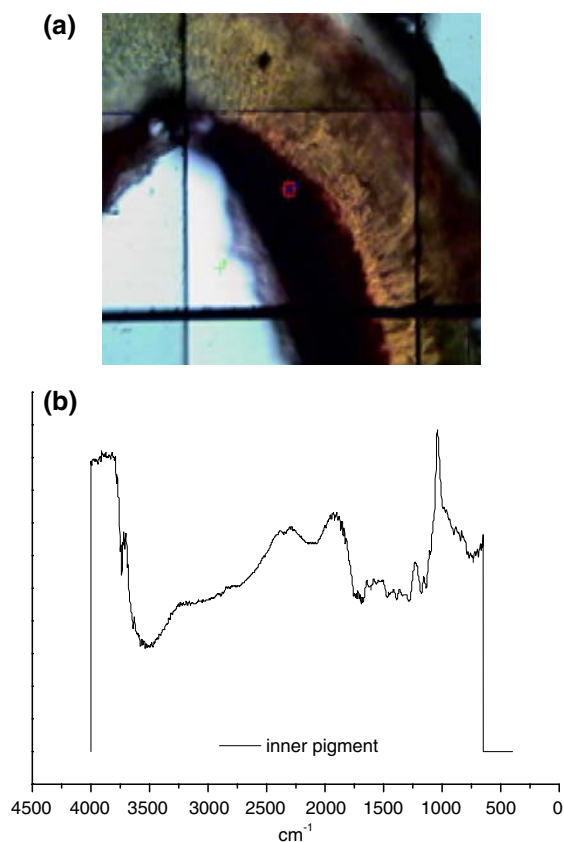


Fig. 4 FT-IR spectra of the inner pigment layer of cotton seed coat. **a** infrared mapping; **b** FT-IR spectra of inner pigment layer of cotton seed coat

1988; Reeves and Valle 1932; Rokhin et al. 1994). The inner pigment layer was analyzed in this study and its FT-IR spectra are shown in Fig. 4b. The rectangular box in Fig. 4a delineates the region selected for infrared mapping.

Bands due to the absorption of lignin in the spectra shown in Fig. 4b are summarized in Table 1. Analysis of those bands indicates that the composition of the inner pigment layer is mainly lignin. Bands at 1,319, 1,234, and 1,260 cm^{-1} are due to the absorption of syringyl unit and guaiacyl unit, respectively, which indicates that syringyl unit (I in Fig. 5) and guaiacyl unit (II in Fig. 5) are the main types in the lignin structure in the CSC inner pigment layer. Tannin is a kind of aromatic polymer which is very similar to lignin in its basic structure units. Besides their similar structure, these two components often exist as a mixture in plants. Therefore, it is difficult to separate tannin from lignin very well by FT-IR

spectra. All aromatic and polyphenolic compounds in the CSC inner pigment layer are considered lignin-like compounds and are called lignins herein.

The color of substances is the results of their absorption in the visible light region. Basic structural units such as guaiacyl unit, syringyl unit, and hydroxyphenyl unit in lignins are propane structures with C=O, C=C, and -OH on their side-chains. In fact these propane structures show no absorption in the visible light region. However, most of the side-chains in lignins, such as C=C, C=S, and C=O, are chromophores and can cause absorption in the visible light region. Therefore, it is mainly the chromophores on the side-chains in lignins that cause the color of the lignins. Rokhin et al. (1994) indicated in their research that CSC is rich in lignins with many free COOH and phenol-OH groups on the side-chains. Those groups can form chromophoric systems and deepen the color of pigmented layers.

In this study, no further analysis was carried out on the CSC colorless layer. According to reports, there are some aromatic compounds lignified before maturity in the cell walls of the CSC colorless layer (Himmelsbach et al. 2003; Ryser et al. 1988). It was reported that carboxylic acid exists in the region between the CSC colorless layer and the upper palisade layer (Himmelsbach et al. 2003; Reeves and Valle 1932).

Conclusions

In the present paper, the chemical composition of CSC was studied. The results showed that CSC consists of epidermis layer, outer pigment layer, colorless layer, palisade layer, and inner pigment layer. According to analysis of FT-IR spectra from each layer of the CSC, the following conclusions can be made. The CSC epidermis is covered with wax and cutin. In addition, cellulose and pectin are the main components of the CSC epidermal layer. Pectin, hemicellulose, and lignins (aromatic and polyphenolic compounds) are the main components of the CSC palisade layer. The inner and outer pigment layers are mainly composed of lignin-like compounds (aromatic and polyphenolic compounds) with polyphenolic-OH on its chain. Cotton seed coat fragments are the most resistant impurities on cotton fabric. Study of the structure and chemical composition of CSC helps to improve studies on its enzymatic degradation.

Table 1 Main bands of cotton seed coat in FT-IR spectra of cotton seed coat

Compound	Group	Absorbance (cm ⁻¹)		
Cellulose	C–OH, C–O–C, C–C, ring	1,161		
		1,112		
		1,060		
		1,031		
		982		
		898		
		Pectic ester	C=O O–CH ₃ –CH ₃	1,741
				1,455
				1,239
		Calcium pectate	Asymmetric, COO ⁻ Symmetric, COO ⁻	1,614
1,425				
Pectic acid	COOH	1,745		
Pectin	C–OH, C–O–C, C–C, C–H, ring	1,151		
		1,004		
		1,082		
		1,052		
		1,022		
		972		
		891		
		Hemicellulose	C=O CH ₃ C–C–O C–OH, C–O–C, C–C, ring	1,732
				1,370
				1,250
1,160				
1,148				
1,108				
1,070				
Wax and cutin	–CH ₂ –CH– C=O –OH Phenolic–OH Aromatic, –H, CH ₂ –CH– C=O Aromatic-skeleton Aromatic-skeleton CH ₃ Aromatic, (C–H) Syringyl, C–O Guaiacyl, C–O Syringyl, C–O C–H	1,040		
		980		
		895		
		811		
		2,923		
		2,885		
		1,732		
		3,136		
		3,345		
		2,929		
Lignin	Phenolic–OH Aromatic, –H, CH ₂ –CH– C=O Aromatic-skeleton Aromatic-skeleton CH ₃ Aromatic, (C–H) Syringyl, C–O Guaiacyl, C–O Syringyl, C–O C–H	2,887		
		1,750–1,660		
		1,602		
		1,519		
		1,460–1,452		
		1,369		
		1,319		
		1,260		
		1,234		
		897, 846		

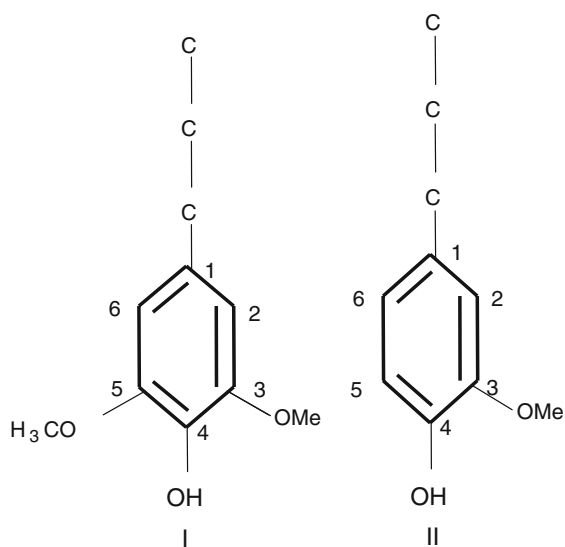


Fig. 5 Structure unit in cotton seed coat lignin. *I* syringyl unit; *II* guaiacyl unit

Therefore, it is expected to improve studies on the removal of cotton seed coat fragments from cotton fabric, as well as the effectiveness of bioscouring. However, the present study deals with analysis of the components of CSC layers, and detailed information about the components and microstructure of CSC layers is needed for study of enzymatic degradation of CSC. Further detailed studies on the components of the CSC layers using immunolabeling and histochemistry will be done to support these conclusions in forthcoming experiments.

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