

## STUDY OF THE GREEN COTTON FIBRES

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### Abstract

Recent investigation of naturally coloured cottons have shown that brown cotton is very similar in morphology to white cotton while green cotton is different since it contains suberin. Suberin containing mainly bifunctional fatty acids can theoretically form a three dimensional network in the presence of glycerol, which is also found in green but not in white cotton. How this three-dimensional network influences the structure of the individual crystallites of cotton cellulose was investigated in this research. To confirm the presence of suberin in the green cotton fibre, infrared spectroscopy measurements were performed. According to the results of infrared spectroscopy, it was found, that  $O_6 - H \dots O_{bridge}$  bond which is normally found in cellulose I, is missing at the spectrum of green cotton. Additionally, two bands at  $700\text{ cm}^{-1}$  and at  $1201\text{ cm}^{-1}$  due to the OH - in plane bending appeared. To get a clear picture concerning the structural differences between the green cotton and the other types, the X-ray diffraction measurements and iodine absorption were carried out. According to the results we found out, that the presence of suberin does not influence the structure of the individual crystallites but hinders the development of the crystallites in the green cotton fibres.

### Introduction

Cotton (*Gossypium*) belongs to a flowering plant grown from seed. There are 39 different wild species of cotton at the present time and four of them have been cultivated and are today of the most commercial importance: new World species *G.hirsutum* and *G.barbadense* and old World species *G.arboretum* and *G.herbaceum*. All 39 species are different in leaf shape, leaf colour, flower, seed, lint, lint colour and length. The lint colour may be white to dirty white, different shades of brown (light brown to chocolate and mahogany red), and green (bright or emerald green which speedily fades to a greenish rusty brown)<sup>1</sup>. The brown cotton is an ancient one. Some researchers claim that today's white cottons are mutants from the native coloured cottons since the majority of white cottons bear coloured lint rather than white. The green cotton is probably a new mutant from the white cotton, first found on a field in Texas. The origin

of the brown and green cottons available today on the cotton market, are varieties of species *G.hirsutum*. Seeds of these cottons are available in seed banks all over the world.<sup>2</sup>

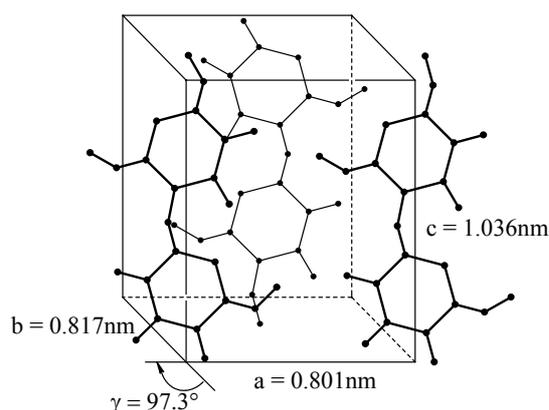
A considerable number of studies have been carried out on white cottons, while coloured cottons were always left behind. However, when pollution started to be the most urgent problem of the world, naturally coloured cottons stand out. White cotton is one of the most chemically intensive crops produced. Although grown on 3 - 5% of the Earth's farmland, it is responsible for the use of 25% of the world's pesticides. For this reason, organically grown cotton has attracted a great deal of attention over the last few years. Brown and green naturally coloured cottons can be grown organically or conventionally. If grown organically this cotton is the most environmentally friendly of all cottons.

The major component of cotton fibres is cellulose as shown in Table 1. The primary wall usually contains besides cellulose other substances as waxes, pectic substances, inorganic salts and a part of the nitrogenous material. The winding layer and the secondary wall are nearly pure cellulose while in the lumen, the pigment, the rest of the protein, inorganic substances, sugar and organic acids have been found.

**Table 1:** Chemical composition of the mature cotton fibre<sup>3</sup>

| Constituent                           | Per cent of dry weight |
|---------------------------------------|------------------------|
| Cellulose                             | 94,0                   |
| Protein (%N * 6.25)                   | 1,3                    |
| Pectic substances                     | 0,9                    |
| Inorganic substances                  | 1,2                    |
| Wax                                   | 0,6                    |
| Malic, citric and other organic acids | 0,8                    |
| Total sugars                          | 0,3                    |
| Other                                 | 0,9                    |
| <b>Total</b>                          | <b>100,0</b>           |

The cellulose chain molecules are bound together by hydrogen bonds. This leads to the formation of a three dimensional monoclinic crystalline lattice shown in figure 1. The crystalline lattice in the figure is that proposed by Sugiyama et al.<sup>4</sup> and has the following crystallographic dimensions:  $a = 0.801$  nm,  $b = 0.817$  nm,  $c = 1.036$  nm,  $\alpha = \beta = 90^\circ$  and  $\gamma = 97.3^\circ$ .



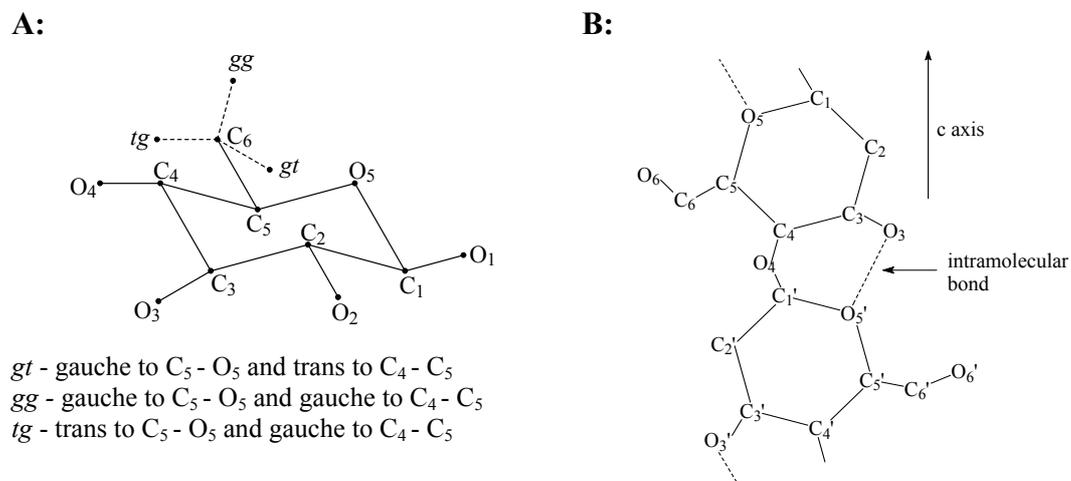
**Figure 1:** Cellulose I unit cell according to Sugiyama et al.

This structure is similar in dimensions to the first lattice proposed by Meyer and Misch (1937) but differs in the designation of the chain axis. The chain axis in the unit cell proposed by Meyer and Misch denoted as the b axis, is more often denoted as the c axis (Sugiyama et al.<sup>4</sup>) in recent years. Consequently, the designation of crystallographic planes in the unit cell of cellulose I is different.

According to Meyer and Misch<sup>6</sup>, cellulose I contains two intramolecular hydrogen bonds:  $O_3-H...O_5'$  and  $O_6-H...O_2'$ . In that case, the orientation of the  $CH_2OH$  groups is parallel and perpendicular to the fibre axis. Later, in 1957, Tsuboi<sup>6</sup> showed from the parallel dichroism of the  $CH_2$  stretching bands that the  $CH_2OH$  groups are orientated just parallel and that an  $O_6-H...O_2'$  bond can be ruled out. This hypothesis was confirmed in the early 1970's by Sarko et al.<sup>7</sup>. They found that the  $CH_2OH$  groups are in the *gauche - trans* conformation and not in the *trans - gauche* as had been suggested by some authors previously<sup>7</sup>. The *gauche - trans* conformation enables just one  $O_3-H...O_5'$  bond, and again an  $O_6-H...O_2'$  bond is ruled out. This is illustrated in table 2 and figure 2.

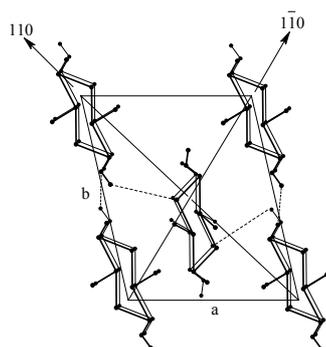
**Table 2:** Dichroism of  $CH_2$  stretching bands

| CH <sub>2</sub> OH conformation | Predicted dichroism of CH <sub>2</sub> stretching bands |              |
|---------------------------------|---|--------------|
|                                 | Symmetrical   | Asymmetrical |
| <i>gt</i>                       | 2.56  | 0.84 ⊥       |
| <i>gg</i>                       | 0.02 ⊥  | 1.83         |
| <i>tg</i>                       | 1.84  | 0.47 ⊥       |



**Fig. 2:** A - the Newman projections of three side-chain conformations, B - the intramolecular bond

Considering the parallel dichroism of the CH<sub>2</sub>OH groups, the C<sub>6</sub> hydroxyl group of one chain may be hydrogen bonded to the bridge oxygen of the next chain in the centre of the cell. One such set of intermolecular bonds can be formed in the  $1\bar{1}0$  and in the 110 plane (figure 3). Another possible set of intermolecular hydrogen bonds is in the 200 plane between the C<sub>2</sub> hydroxyl group of one chain and the C<sub>6</sub> oxygen of another <sup>6</sup>.



**Fig. 3:** Intermolecular bonds in Cellulose I

The research, from which some of the results are described in this article, was started in 1995 at Bolton Institute, UK in collaboration with Department of Textiles,

University of Ljubljana. After this research is finally concluded, some interesting results were obtained at the area of green cotton fibre structure.

## Experimental

### Materials

In this research, five cottons from the 1994/95 crop were used: American white Memphis cotton - conventionally grown cotton, American brown cotton - naturally coloured cotton, American green cotton - naturally coloured cotton, Israeli brown cotton - naturally coloured cotton, Israeli green cotton - naturally coloured cotton.

The Israeli brown and green naturally coloured cottons, which were used in this research, were examined for some structural and general properties, i.e. properties where the coloured cottons were significantly different compared to the white sample.

The cotton samples were tested as “raw material”. For some experiments samples were subjected to Soxhlet extraction with ethanol for 24 hours in order to remove waxes, oils and protoplasmic residue. In that case, samples are denoted as “extracted with ethanol”.

### Infrared spectroscopy

The infrared spectra of the samples were measured with a Mattison 3000 FTIR Spectrophotometer. The samples were prepared by the standard procedure recommended by O'Connor et al.:<sup>10</sup> powdered sample (2.5 mg) is mixed with 300 mg of anhydrous potassium bromide, and the mixture compressed into a KBr disc. All discs were stored in a desiccator until the spectrum was recorded.

The infrared measurements in polarised light were carried out at UMIST, Manchester with the fibres laid parallel or perpendicular to the polarised light.

For better understanding the results of this research, the main features in the cellulose I spectrum are quoted:

1. The strong parallel band at  $3350\text{ cm}^{-1}$  indicates an intramolecular bond, possibly of the type  $\text{O}_3 - \text{H} \dots \text{O}_5'$ . The perpendicular bands at  $3305$  and  $3405\text{ cm}^{-1}$  indicate intermolecular hydrogen bonds between chains, possibly of the  $\text{O}_6 - \text{H} \dots \text{O}_{\text{bridge}}$  type.

- The other set of intermolecular bonds of the  $O_2 - H \dots O_6$  type, has not been identified. It is possible that bands, which belong to this set of intermolecular bonds, overlap with some others on the higher frequency side of the  $3350 \text{ cm}^{-1}$ .<sup>6,8,9</sup>
2. The  $\text{CH}_2$  symmetric stretching frequency at  $2853 \text{ cm}^{-1}$  and the  $\text{CH}_2$  symmetric bending frequency at  $1430 \text{ cm}^{-1}$  are both parallel, which rules out the possibilities of an intramolecular hydrogen bond involving the  $\text{C}_6$  hydroxyl group.<sup>6</sup>
  3. Bands reduced in intensity after deuteration at  $1205$ ,  $1336$ , and  $1455 \text{ cm}^{-1}$  correspond to the OH in-plane bending vibrations. Frequencies at  $663$  and  $700 \text{ cm}^{-1}$  are assigned to the OH out-of plane bending vibrations.<sup>8</sup>
  4. According to infrared study of deuterated sugars, the  $\text{C}_1 - \text{H}$  stretching and bending vibrations of cellulose I are assigned to the  $2914$  and  $1358 \text{ cm}^{-1}$  bands.<sup>6</sup>
  5. The strong parallel band at  $1162 \text{ cm}^{-1}$  may be assigned to the asymmetric COC bridge stretching frequency, from the infrared spectra of a number of celluloses and their derivatives.<sup>8-10</sup>
  6. The ring stretching frequencies are assigned to the band at  $895 \text{ cm}^{-1}$  (asymmetric out - of - phase) and at  $1110 \text{ cm}^{-1}$  (asymmetric in - phase), while the band near  $800 \text{ cm}^{-1}$  is assigned to the ring breathing vibration.<sup>8,9</sup>
  7. The bands between  $985$  and  $1058 \text{ cm}^{-1}$  arise from the C - OH stretching modes.<sup>8,9</sup>

### Wide angle X-ray diffraction

Wide angle X-ray diffraction analysis of the samples was carried out at the Institute of Physical Chemistry, University of Graz. The unit cell and crystallite dimensions, and the crystallinity index of samples were obtained from the data.

The samples were investigated by means of a two - circle goniometer, using Ni filtered copper radiation ( $\text{Cu K}_\alpha$ ,  $\lambda = 1.542 \text{ \AA}$ ) and a linear position sensitive detector. Measurements were performed in two separate runs, with the detector placed at a scattering angle of  $20^\circ$  and  $40^\circ$ , respectively, thus delivering information on the scattering behaviour in a wide range of scattering angles ( $2\theta \approx 9$  to  $50^\circ$ ). During the measurement, each sample was rotated around the primary beam in steps of  $5^\circ$  (total rotation by  $180^\circ$ ) and for each step an intensity vs. scattering angle curve was measured.

From the angular positions of the resolved peaks the  $d$  - spacing ( $d_{hkl}$ ) were calculated according to Bragg's equation<sup>12</sup>

$$d_{hkl} = \frac{\lambda \cdot n}{2 \sin \theta}, \quad (1)$$

where  $\lambda$  is the wavelength of the x-ray,  $n$  is the ordinal number of the reflection and takes in accordance with the order of the corresponding reflection, the values 1, 2, 3, etc., and  $\theta$  is the scattering angle in radians. From the half widths of the peaks (corrected for the instrumental broadening) the crystallite dimensions  $L_{hkl}$  were calculated by means of Scherrer's equation<sup>13</sup>

$$L_{hkl} = \frac{K\lambda}{d(2\theta) \cos \theta}, \quad (2)$$

where factor  $K$  is 0.9 and  $d(2\theta)$  is the width of the peak or more correctly the integral breadth in radians. From the crystallite dimensions  $L_{hkl}$  a crystallinity index was determined as suggested by Krässig<sup>14</sup>

$$CrI = \frac{1 - h_{am}}{h_{cr}} = \frac{1 - h_{am}}{h_{tot} - h_{am}}, \quad (3)$$

where  $h_{cr}$  is crystalline height and  $h_{am}$  is amorphous height of the 200 reflection.

In nature, the algal - bacterial and cotton - ramie types of cellulose can be found. The difference is that the former contains mainly the cellulose I $\alpha$  which has a triclinic unit cell while the latter contains mainly the cellulose I $\beta$  which has a monoclinic unit cell. To extract specific feature from each structure, the use of the discriminant function ( $Z$ ) between the two types is recommended by Wada et al.<sup>15</sup>

$$Z = 1364 d_{(1\bar{1}0)} - 1325 d_{(110)} - 148 d_{(012)(102)} + 1578 d_{(200)} + 3566 d_{(023)(004)} - 1606, \quad (4)$$

where  $Z > 0$  means algal - bacterial type (I $\alpha$  triclinic structure) and  $Z < 0$  means cotton - ramie type (I $\beta$  monoclinic structure).

### Iodine absorption

The iodine absorption is a classic method proposed by Schwertassek.<sup>16</sup> In this method, iodine is absorbed from aqueous solution, which contains potassium iodide in order to increase the solubility of iodine, by the formation of triiodide ions.

The cotton sample (0.3 g) is mixed with the iodine solution and saturated sodium sulphate and left for 1 hour in the dark. The iodine remaining in the solution is titrated with 0.02 M sodium thiosulphate and the Iodine Sorption Value (ISV) in mg I<sub>2</sub> absorbed per 1g of sample is calculated as follows<sup>16</sup>

$$ISV = \frac{(b-t) \cdot (M \cdot 102) \cdot (M \cdot 126.91)}{w} = \frac{(b-t) \cdot 2.04 \cdot 2.54}{w}, \quad (5)$$

where  $b$  is the volume of sodium thiosulphate in millilitres for blank titration,  $t$  is the volume of sodium thiosulphate in millilitres for the titration of the sample solution,  $M$  is the molarity of the sodium thiosulphate, 102 is a total volume of the solution in ml, and  $w$  is the accurate dry weight of the cotton sample in grams.

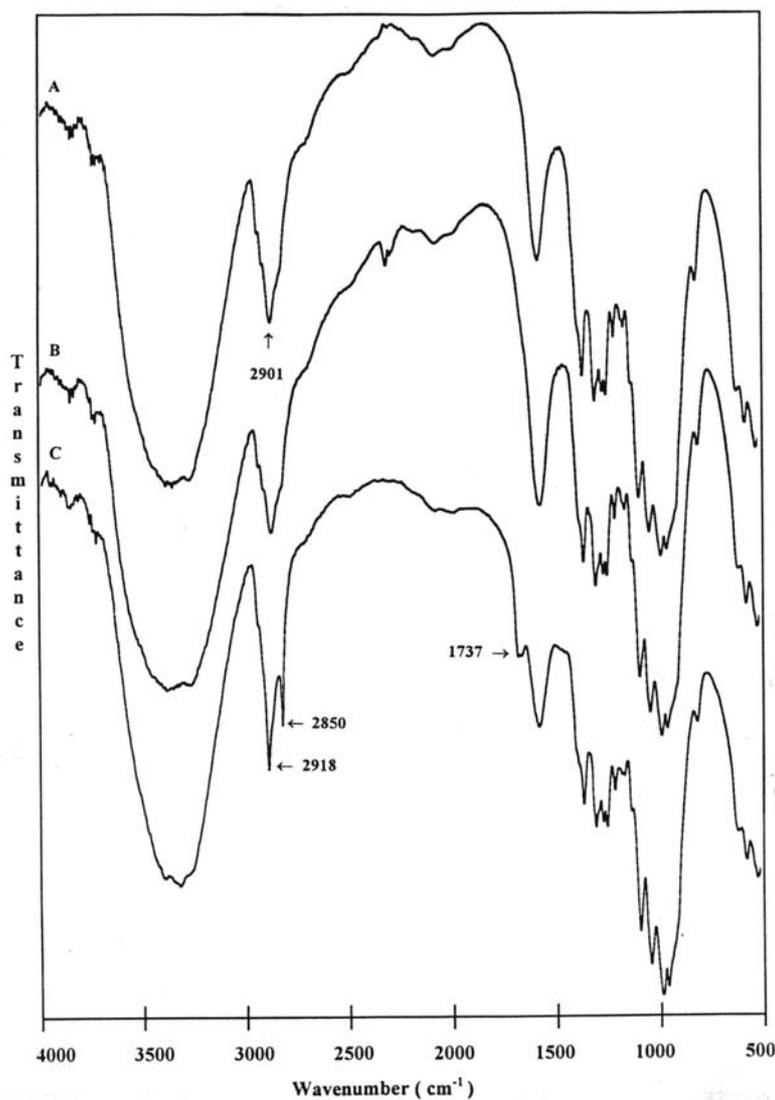
According to Schwertassek, the absorption takes place in the amorphous phase. A ratio of ISV per g cellulose to 412 (mg iodine absorbed per 1g of methyl cellulose) gives a value for the amorphous fraction. The percentage crystallinity is calculated using equation (6)<sup>16</sup>

$$Percentage\ crystallinity = 100 - \left( \frac{ISV}{412} * 100 \right). \quad (6)$$

## Results and discussion

### Infrared spectroscopy

From the infrared spectra (figure 4) can be seen that both the white and brown cotton spectra have similar bands.



**Figure 4:** Spectra of A – white, B – brown and C – green cotton samples

The green cotton spectrum has:

- an additional band at  $700\text{ cm}^{-1}$  (OH - out of plane bending),
- an additional band at  $1201\text{ cm}^{-1}$  (OH - in plane bending),
- an additional band at  $1737\text{ cm}^{-1}$  (C = O stretching typical for ester),
- an additional band at  $2850\text{ cm}^{-1}$  ( $\text{CH}_2$  symmetrical stretching),
- a missing band at  $2894\text{ cm}^{-1}$  (C - H stretching),
- an additional band at  $2918\text{ cm}^{-1}$  (C - H stretching), and

- a missing band at  $3305\text{ cm}^{-1}$  (OH stretching which belongs to the intermolecular hydrogen bond  $\text{O}_6\text{-H} \dots \text{O}_{\text{bridge}}$  in  $\bar{1}\bar{1}0$  crystallographic plane).

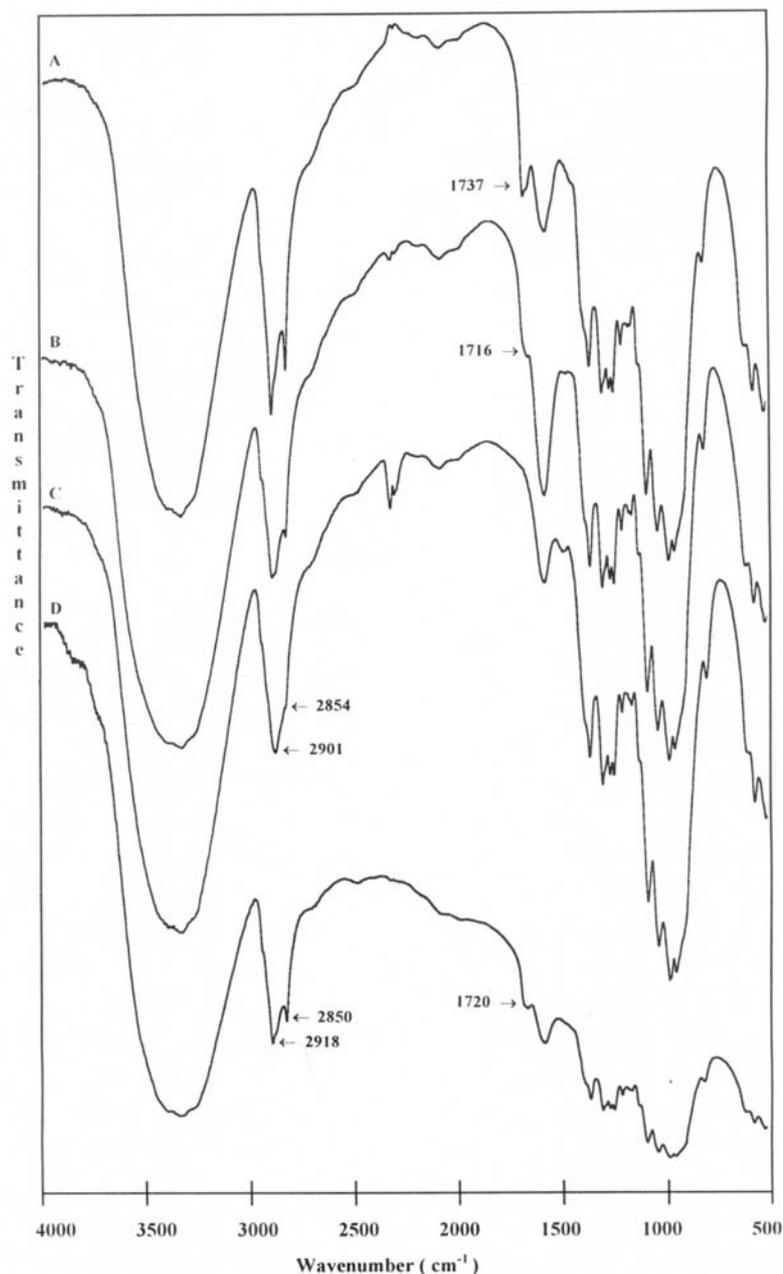
The three bands at  $1737$ ,  $2850$  and  $2918\text{ cm}^{-1}$  can be attributed to the wax since

- wax as an ester of long chain fatty acids and alcohols gives a typical  $\text{C} = \text{O}$  stretching frequency in the absorption range from  $1736 - 1750\text{ cm}^{-1}$ , and
- its long molecules give  $\text{CH}_2$  symmetrical stretching and  $\text{C-H}$  stretching in the absorption range from  $2840 - 2860\text{ cm}^{-1}$  and from  $2700 - 3400\text{ cm}^{-1}$ , respectively.

To confirm, that these three bands were due to wax, some additional experiments, were performed. In the first experiment, the green cotton was extracted with ethanol for 24 hours (esterification). From Figure 5B it can be seen that the band at  $1736\text{ cm}^{-1}$  disappears, while a new band at  $1716\text{ cm}^{-1}$  appears. The new band can be attributed to the  $\text{C} = \text{O}$  stretching from the carboxylic acid for which the absorption range is quoted as from  $1700 - 1725\text{ cm}^{-1}$ . The bands at  $2918$  and  $2850\text{ cm}^{-1}$  are reduced in intensity because the proportion of  $-\text{C}-\text{H}$  groups, which belong to the wax, is reduced by ethanol extraction.

The second experiment was scouring with 3 % sodium hydroxide of the ethanol extracted sample (alkali - catalysed hydrolysis). From Figure 5C it can be seen that after ethanol extraction and scouring, the band at  $2918\text{ cm}^{-1}$  disappears while the band at  $2850\text{ cm}^{-1}$  is significantly reduced in intensity. The band at  $1736\text{ cm}^{-1}$  disappears, while a new band at  $1556\text{ cm}^{-1}$  appears due to the carboxyl ions arising from the alkali - catalysed hydrolysis. The normal absorption range of the  $\text{C} = \text{O}$  stretching frequency from carboxyl ions is between  $1550 - 1610\text{ cm}^{-1}$ .

In the third experiment the ethanol extracted sample was treated with 1% HCl. From the Figure 5D can be seen that the bands at  $2918$  and  $2850\text{ cm}^{-1}$  are still present while the band at  $1737\text{ cm}^{-1}$  disappears. A new band at  $1722\text{ cm}^{-1}$ , which appears in the spectrum, can be attributed to the carbonyl stretching from the carboxylic acid groups (acid - catalysed hydrolysis).



**Figure 5:** Spectra of green cotton sample A – untreated, B – ethanol extracted, C – ethanol extracted and scoured with 3 % NaOH, D – ethanol extracted and treated with 1 % HCl

From these results it was concluded, that the bands at 1737, 2850 and 2918  $\text{cm}^{-1}$  in the spectrum of green cotton were due to the wax, since the changes in the spectrum after alkali and acid hydrolysis are to be expected. Ethanol extraction and scouring are expected to remove all the impurities from cotton. The spectra of the green cotton after

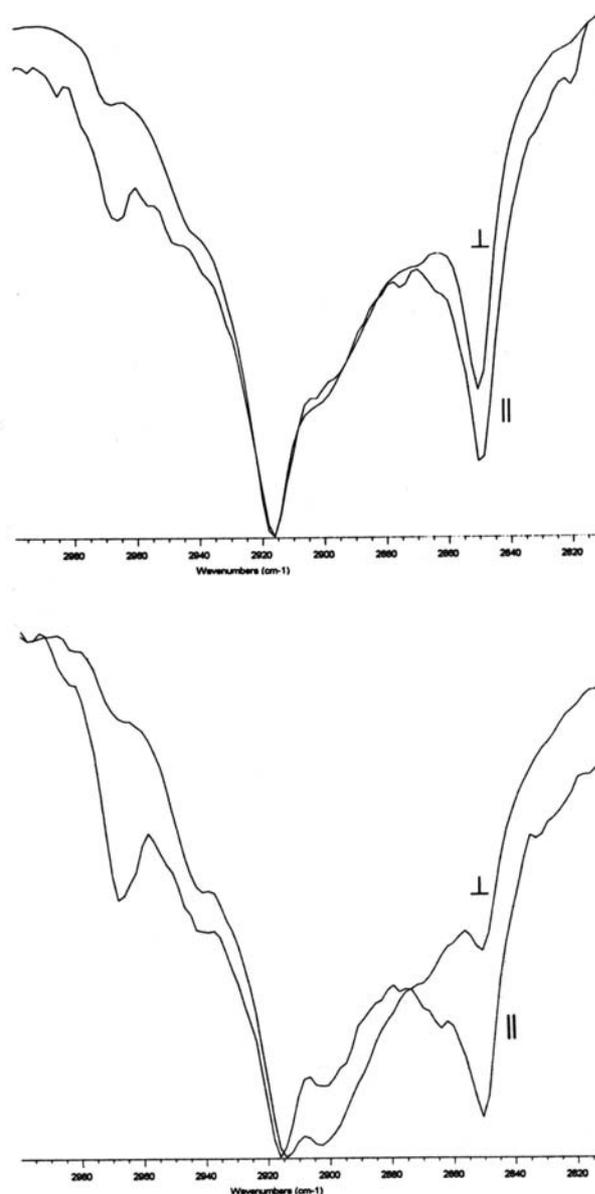
purification by both ethanol extraction and scouring (Figure 5C and 5D) show no carbonyl ester stretching band because the wax has been totally removed.

Although, these three bands in the spectrum of the green cotton are resolved, there are some differences which are not visible in the spectrum but which appear in the analysis of the peaks. There are two additional bands at  $700\text{ cm}^{-1}$  due to the OH - out of plane bending and at  $1201\text{ cm}^{-1}$  due to the OH - in plane bending, and one band at  $3305\text{ cm}^{-1}$  is missing. This can be attributed to the OH stretching of the intermolecular hydrogen bond  $\text{O}_6\text{-H} \dots \text{O}_{\text{bridge}}$  in the  $\bar{1}10$  crystallographic plane. Two additional bands disappear and one missing band appears after the green cotton is extracted with ethanol. All three bands are connected with the  $\text{CH}_2\text{OH}$  group of cellulose I.

As described before, the  $\text{CH}_2\text{OH}$  group in cellulose I usually has *gauche* - *trans* conformation although different arrangements are possible. Figure 6 shows the polarised infrared spectra of an untreated sample at the green cotton and one that has been ethanol extracted and scoured.

It can be seen, that in the green raw cotton, the *gauche* - *trans* conformation is inhibited because the bands at  $2850$  and  $2918\text{ cm}^{-1}$  show no dichroism. The dichroism of these two bands appears after extraction and scouring. Since the missing intermolecular band appears and the bands attributed to the OH - in plane and out - of plane bending disappear after extraction, all these bands could be due to the same cause.

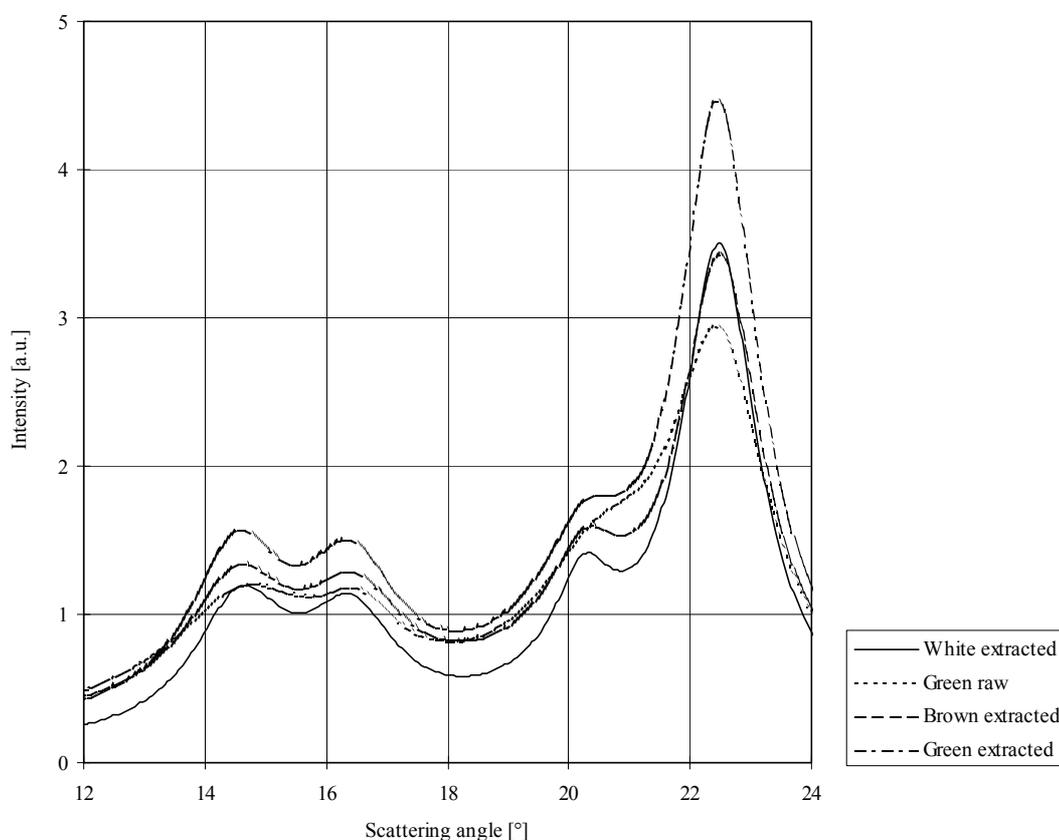
Yatsu et al.<sup>17</sup> showed that natural green cottons contain suberin a wax like material and that the suberin exists between the layers of cellulose in the secondary cell wall of the fibre. Suberin is different to normal cotton wax in that one of the components is an  $\omega$  - hydroxy carboxylic acid: hence suberin contains an aliphatic polyester. The polymeric nature is likely to explain why it is more difficult to remove from the fibre than normal cotton wax. Ethanol extraction for 24 hours or a combination of ethanol extraction and sodium hydroxide scouring were needed for complete removal. The presence of the suberin layers may distort the cellulose I structure during fibre formation and lead to the absence of the  $\text{O}_6\text{-H} \dots \text{O}_{\text{bridge}}$  bond which is normally found in cellulose I. The two additional bands at  $700\text{ cm}^{-1}$  and at  $1201\text{ cm}^{-1}$  due to the OH - in plane bending may also be present at the spectrum due to the distortion of the structure.



**Figure 6:** Spectra of green cotton sample; above – untreated, below – ethanol extracted and scoured with 3 % NaOH

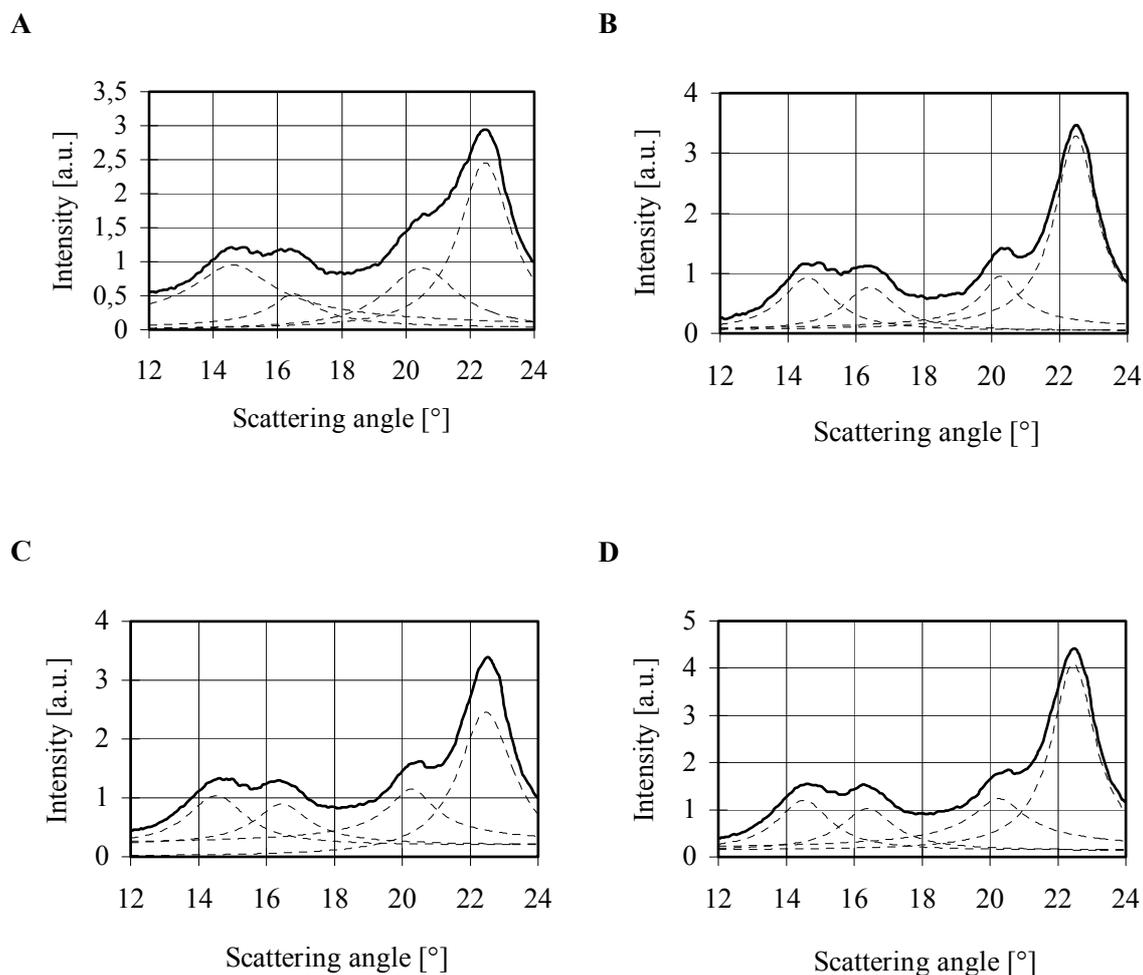
### X-ray diffraction

To get a clear picture concerning the structural differences between the green cotton and the other types, the X-ray diffraction measurements were carried out. The samples tested by X-ray diffraction were the green raw cotton, and the white, brown and green ethanol extracted cottons. The full scattering curves of the samples are given in the Figure 7.



**Figure 7:** Scattering curves of the green raw cotton, and the white, brown and green ethanol extracted cottons, shown in the full angular range

From the diagram it can be seen that the samples show different scattering patterns in the angular range below  $27^\circ$  with regard to the height and the shape of the peaks. In order to get a quantitative characterisation of the differences, each scattering curve was approximated by a set of overlapping Pearson VII functions in the  $2\theta$  range from  $12$  to  $24^\circ$ . The curves obtained are presented in Figure 8.



**Figure 8:** Scattering curve of A - green raw cotton, B – white, C – brown, D – green cotton ethanol extracted

The results of the  $d$ -spacing, crystallite dimensions  $L_{khl}$ , the discriminant functions  $Z$  and the crystallinity indices  $CrI$  are listed in Table 3. From the table it can be seen that all four samples have negative discriminant values, which indicate monoclinic structures. The crystallinity values in all extracted cottons are almost the same. In the case of green raw cotton, the value is much lower but after the extraction it increases. This contradicts normal expectations.

**Table 3:** Wide-angle X-ray diffraction

| Sample                  | Peak position [°] | hkl         | $d_{hkl}$ [nm] | $L_{hkl}$ [nm] | Z    | CrI  |
|-------------------------|-------------------|-------------|----------------|----------------|------|------|
| White ethanol extracted | 34.43             | 023 004     | 0.261          |                | -4.3 | 0.64 |
|                         | 14.58             | $\bar{1}10$ | 0.608          | 4.85           |      |      |
|                         | 16.42             | 110         | 0.540          | 5.03           |      |      |
|                         | 20.25             | 012 102     | 0.439          | 5.91           |      |      |
|                         | 22.48             | 200         | 0.396          | 6.29           |      |      |
| Brown ethanol extracted | 34.48             | 023 004     | 0.260          |                | -1.1 | 0.60 |
|                         | 14.52             | $\bar{1}10$ | 0.610          | 4.35           |      |      |
|                         | 16.47             | 110         | 0.538          | 4.51           |      |      |
|                         | 20.25             | 012 102     | 0.429          | 4.31           |      |      |
|                         | 22.49             | 200         | 0.395          | 6.01           |      |      |
| Green ethanol extracted | 34.48             | 023 004     | 0.260          |                | -0.4 | 0.62 |
|                         | 14.52             | $\bar{1}10$ | 0.611          | 4.82           |      |      |
|                         | 16.47             | 110         | 0.541          | 4.84           |      |      |
|                         | 20.25             | 012 102     | 0.439          | 3.65           |      |      |
|                         | 22.49             | 200         | 0.396          | 6.23           |      |      |
| Green raw               | 34.48             | 023 004     | 0.260          |                | -3.6 | 0.56 |
|                         | 14.52             | $\bar{1}10$ | 0.606          | 3.47           |      |      |
|                         | 16.47             | 110         | 0.537          | 5.42           |      |      |
|                         | 20.25             | 012 102     | 0.434          | 3.12           |      |      |
|                         | 22.49             | 200         | 0.396          | 5.59           |      |      |

The  $d$  - spacing values for all four samples are very close, which indicates very similar unit cells, volumes and densities. The unit cells dimensions, calculated from the  $d$  - spacing values are presented in Table 4, in which the density value represents the density of the crystalline regions.

**Table 4:** Unit cell dimensions

| Sample                  | a [nm] | b [nm] | c [nm] | $\gamma$ [°] | V [nm <sup>3</sup> ] | $\rho$ [g.cm <sup>-3</sup> ] |
|-------------------------|--------|--------|--------|--------------|----------------------|------------------------------|
| White ethanol extracted | 0.79   | 0.83   | 1.04   | 96.8         | 0.682                | 1.570                        |
| Brown ethanol extracted | 0.79   | 0.84   | 1.04   | 97.1         | 0.682                | 1.568                        |
| Green ethanol extracted | 0.79   | 0.84   | 1.04   | 97.0         | 0.687                | 1.558                        |
| Green raw               | 0.79   | 0.83   | 1.04   | 96.9         | 0.677                | 1.581                        |

From Table 4 it is evident, that all four samples have very similar unit cell dimensions. Consequently the structural differences in the fibres cannot be explained by variations in the unit cell dimensions.

A main difference among the samples can be found in the crystallite dimensions  $L_{\text{hkl}}$  shown in Table 5. Assuming, that the crystallites in all four cottons have a monoclinic structure, the crystallite dimensions  $a$ ,  $b$ ,  $c$ , and  $\gamma$  can be evaluated in the same way as the unit cell. The results are presented in Table 5.

**Table 5:** Crystallite dimensions

| Sample                  | a [nm] | b [nm] | c [nm] | $\gamma$ [°] | V [nm <sup>3</sup> ] |
|-------------------------|--------|--------|--------|--------------|----------------------|
| White ethanol extracted | 6.29   | 5.91   | 6.50   | 93.0         | 312.0                |
| Brown ethanol extracted | 6.01   | 4.31   | 4.59   | 86.8         | 144.5                |
| Green ethanol extracted | 6.23   | 3.89   | 3.79   | 89.8         | 150.8                |
| Green raw               | 5.59   | 3.12   | 3.25   | 63.2         | 74.8                 |

From the table it can be seen, that before extraction, the green cotton has smaller crystallites than the other types. After extraction these crystallites become larger with a volume similar to those of the brown cotton. The crystallinity index increases, which means that further crystallisation occurs during the ethanol extraction of the green cotton; the removal of the suberin may release the strains in the structure. The results of the iodine absorption test show a similar trend.

### Iodine absorption

The results of iodine absorption test are shown in Table 6.

**Table 6:** Fibre accessibility (ISV - iodine sorption value)

| Sample        | ISV [mg I <sub>2</sub> .g <sup>-1</sup> sample] |                   | Degree crystallinity [%] |                   |
|---------------|---|-------------------|--------------------------|-------------------|
|               | raw   | ethanol extracted | raw                      | ethanol extracted |
| White Memphis | 36.3  | 67.0              | 91.2                     | 83.7              |
| Brown Cotton  | 89.8  | 104.5             | 78.2                     | 74.7              |
| Green Cotton  | 81.2  | 71.3              | 80.3                     | 82.7              |

Results show that the white raw cotton has a significantly lower ISV and hence a higher crystallinity than the brown and green cottons. After ethanol extraction, the ISV of the white and the brown cotton increases while in the case of the green cotton, ISV

decreases. The raw green cotton has thus a more open and less crystalline structure with more interfibrillar surfaces.

### Conclusions

According to Yatsu et al.<sup>17</sup> and Schmutz et al.<sup>19</sup> the green cotton fibres have a different morphology i.e. the secondary cell wall in the green cotton fibre is composed of alternate cellulose - suberin layers. Qualitatively, suberin in the green cotton fibres was identified by infrared spectroscopy. The spectrum of the green cotton has three additional bands which may be attributed to the suberin. Due to its polymeric nature, suberin is more difficult to remove from the fibre than normal cotton wax. Thus, 24 hours extraction with ethanol was required for the complete removal of the suberin.

The influence of suberin layers to the structure of the secondary cell wall of the green cotton fibres was not fully quantified in the previous researches of Yatsu et al.,<sup>17</sup> Elsner,<sup>18</sup> Schmutz et al.<sup>19</sup> and Kolattukudy et al.<sup>20</sup> In this research iodine absorption and X - ray diffraction measurements were carried out. From the iodine absorption results it can be seen that for the white and the brown cottons, accessibility increases and the observed crystallinity decreases after the fibres were extracted with ethanol, while for the green cotton fibres, the reverse occurs. The results obtained by X - ray diffraction (crystallinity index) show a similar trend. From Table 4 it can be seen that the unit cells for all samples are very similar, while the crystallite's dimensions are different (Table 5). Before extraction, the green cotton fibres have small crystallites and a low crystallinity index. After extraction, the crystallites become larger and similar in size to those of the brown cotton fibres.

From results it has been concluded, that the presence of suberin does not influence the structure of the individual crystallites but hinders the development of the crystallites in the green cotton fibres. After ethanol extraction or sodium hydroxide scouring, the removal of the suberin releases the strains in the small crystallites and they coalesce into larger ones (further crystallisation occurs).

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### Povzetek

Novejše raziskave naravno obarvanega bombaža so pokazale, da je rjavi bombaž morfološko zelo podoben belemu bombažu, medtem ko je zeleni različen zaradi vsebnosti suberina. Suberin vsebuje v glavnem bifunkcionalne maščobne kisline, ki teoretično lahko tvorijo tridimenzionalno strukturo, če je prisoten glicerol. Le-ta je bil najden v zelenem ne pa tudi v belem bombažu. Raziskan je bil vpliv te tridimenzionalne strukture na strukturo kristalinitov bombažne celuloze. Prisotnost suberina v zelenem vlaknu je bila potrjena s pomočjo infrardeče spektroskopije. Glede na rezultate je bilo ugotovljeno, da vez  $O_6 - H - O_{most}$ , ki je običajno prisotna v celulozi I, na spektru zelenega bombaža manjka. Dodatno pa sta se pojavila dva nova spektralna trakova pri  $700\text{ cm}^{-1}$  in  $1201\text{ cm}^{-1}$ , ki ustrezata nihanju skupine OH v ravnini. Da bi si bolj natančno razložili ugotovljene strukturne spremembe zelenega bombaža napram belemu in rjavemu, sta bili izvedeni širokokotna rentgenska analiza in metoda jodove absorpcije. Iz rezultatov je bilo ugotovljeno, da prisotnost suberina ne vpliva na strukturo posameznih kristalinitov temveč zavira njihovo rast v zelenem bombažnem vlaknu.