

The Influence of Different Pretreatments on the Quantity of Seed-coat Fragments in Cotton Fibres

Abstract

Cotton combing noil, which is used for production of sanitary wadding, was pretreated with pectinases, cellulases and a combination of both enzymes, and subsequently bleached with hydrogen peroxide or peracetic acid. For comparison the fibers were also alkaline scoured and bleached with both oxidants. The influence of the different processes on the amount of seed-coat fragments and on their further elimination was investigated. The amount of pectin, ash and calcium on fibres, water absorbency, damage to fibres and the degree of whiteness and lightness were examined for differently pretreated fibres. The results show that enzymes have positive influence on the mechanical removal of impurities from cotton fibres; however, after the treatment, noncellulosic substances, especially seed-coat fragments, remain on the fibres. The total amount of impurities is reduced by further treatment with peracetic acid. But to meet pharmacological standards, treatment with hydrogen peroxide is required. However, the results show that less pretentious material could be pretreated using alternative methods.

Key words: cotton, enzymes, bleaching, scouring, seed-coat, combing.

Introduction

Preparation and bleaching are among the most energetically and chemically intensive steps of cotton finishing. The application of enzymes can reduce or even replace the harsh chemicals in some processes of fibre preparation [1]. It was proven that caustic alkaline scouring of cotton can in many cases be replaced by bioscouring with pectinases [2-10]. The problems in both processes are caused by seed-coat fragments.

Seed-coat fragments are part of a seed coat that has been broken from the surface of either mature or immature seeds during mechanical processing. They are usually black or dark brown [11]. Seed-coat fragments in cotton fabric spoil its aesthetic effect, because they differ in colour and morphology from cellulose. Seed-coat fragments are the most resistant impurities of cotton. High amounts of seed-coat fragments are removed by hackling or combing. To eliminate the remaining seed-coat fragments, more concentrated chemical solution and longer steaming are needed than when removing other impurities from raw cotton. Additional expense, higher chemical consumption and a risk of cellulose degradation are the consequences. In practice, not all seed-coat fragments are removed, and those remaining are only bleached with oxidants. [12]

Himmelsbach et al. [13] used a FTIR microscopic mapping and staining reactions on various regions of cotton seeds to elucidate chemical-structural rela-

tionships in the fibre-seed coat regions. Microscopic studies revealed three regions of the fibre base located in modified epidermis that produces cotton fibre: elbow, shank and foot.

The break point of the fibre from the seed is at or just above the outer surface of the epidermis, which is near the elbow. The shank is constricted and in close contact with immediate epidermal cells. What is more, the foot forms an anchor near the inner side of the epidermis. The base of the foot is in contact with the outer pigment layer of the seed.

Electron microscopy indicated that the primary wall at the elbow region has modified chemistry and that cotton fibre has an outer layer of de-esterified pectin and extensin as well as an inner layer consisting of xyloglucans and cellulose. Since the primary wall has considerable amounts of noncellulosic material, studies suggest that other materials (i.e. sugars, proteins, waxes, aromatics) could contribute to the strength of the fibre-seed bond. The outer pigment layer of the seed and the feet of the fibre contain higher amounts of pectate salts and other uronate salts. They suggest that it is possible that pectins contribute to the anchoring of fibres into the outer pigment layer, and thus treatment with calcium chelators in conjunction with pectinolytic enzymes or treatment with hemicellulases could facilitate the separation of fibre bases from the seed-coat.

Csizar and coworkers [11, 14, 15] used cellulases to investigate the effects of

enzymatic treatment on the penetration of chemical solution into seed-coat fragments. They found out that seed-coat fragments are partially degraded by cellulases. The higher the concentration of cellulase, the higher the rate of seed-coat fragment degradation is, until a certain limit is reached. They assumed that cellulase enzyme complex made residual seed-coat fragments more accessible to chemicals. Consequently, it enhances the penetration of alkaline scouring solution and increases the alkaline degradation of seed-coat fragments. Cellulase pretreatment also allowed a reduction in hydrogen peroxide consumption in the consecutive chemical bleaching step.

Since the positive effect of cellulases on the removal of seed-coat fragments was confirmed [11, 14] but the effect of pectinases was only predicted [13], we intended to treat cotton fibres loaded with seed-coat fragments with cellulases, pectinases and their combination. The influence of enzymatic treatment on the seed-coat fragments and on their removal in further mechanical and chemical treatments was investigated. Combing on a Shirley analyser represented the mechanical treatment and bleaching the chemical treatment. The enzymatic treatments were compared to conventional alkaline scouring.

Since seed-coat fragments cannot be removed in a scouring process, further bleaching with hydrogen peroxide (HP) is a common practice [16a]. Peracetic acid (PAA) is also a strong oxidising agent, which is not widely used for

Table 1. The list of processes and their codes.

Code	Treatment process
R	Raw fibers
E	Enzymatic treatment
E0	reference
Ec	cellulase
Ep	pectinase
Ecp	cellulase and pectinase
A	Alkaline scouring
PAA	Bleaching with peracetic acid
HP	Bleaching with hydrogen peroxide
E+PAA	Enzymatic treatment and bleaching with PAA
E+HP	Enzymatic treatment and bleaching with HP
A+PAA	Alkaline scouring and bleaching with PAA
A+HP	Alkaline scouring and bleaching with HP

textile bleaching. PAA is able to attack hard oxidising substances and is used for bleaching fibres, which contain high amounts of lignin. Bleaching optimally takes place at temperatures between 40 °C and 80 °C and at pH 7 to pH 8. Stabilising agents are not needed since almost no fibre damage is caused at these conditions. Peracetic acid decomposes into oxygen and acetic acid and is therefore environmentally safe [17 - 24].

The goal of our work was also to investigate if the bleaching of differently pretreated fibres with PAA could be an alternative to bleaching with HP, regarding the amount of seed-coat fragments and other properties of cotton fibres.

The material investigated was cotton combing noil, which contains a lot of seed-coat fragments and other impurities. Cotton noil is the basis for producing sanitary wadding. Therefore, we observed the properties required for this material, prescribed by European Pharmacopoeia [25]; although achievement of these properties was not our primary goal.

Materials and methods

Materials

Short staple cotton combing noil, consisting of short raw cotton fibres separated out by combing in the production of worsted yarn (waste short fibres, with a substantial quantity of immature fibres and noncellulosic impurities), was obtained from Tosama, Slovenia, where it is used for the manufacturing of wadding.

Pectinolytic enzyme NS 29048 and cellulolytic enzyme Cellusoft L were

obtained from Novozymes, Denmark. The optimal pH for both enzymes is 4.8 with a temperature of 55 °C. Persan S15 – 15% solution of equilibrium peracetic acid (PAA) and 35% hydrogen peroxide, were obtained from Belinka Perkemija, Slovenia. Lavotan RWS (polyoxyethyl-ened fatty alcohol with alcoxylates - a nonionic wetting agent) and Cottoblanc HTD-N (modified alkane sulphonates with sequestering agents: anionic wetting, dispersing and complexing agents) were obtained from CHT, Germany. The above products were of commercial grade and used without further purification.

Sodium acetate from Carlo Erba, Italy, acetic acid and sodium carbonate from Riedel-de Haën, Germany, tetrasodium pyrophosphate from Kemika, Croatia and sodium hydroxide from Šampionka, Slovenia were of analytical grade.

Procedures

Cotton fibres were scoured with pectinases, cellulases and their combination. The fibres were scoured with sodium hydroxide as well. The scoured fibres were subsequently bleached with peracetic acid or with hydrogen peroxide. The list of procedures and their codes is presented in Table 1.

All treatments were done in a JET laboratory dyeing apparatus - Färbeaparät JFL, W. Mathis, at a liquor to substrate ratio of 1:20. The initial weight of all the samples was 200 g. The material was kept in a conditioned chamber at 20 °C and 65% humidity before weighting. Demineralised water was used in all scouring treatments and in bleaching with PAA. Bleaching with HP was done in tap water. The enzymatic treatments were performed at a pH of 4,8 (0,05 M sodium-acetate – acetic acid buffer). The recipes and treatment conditions are presented in Table 2. Sodium pyrophosphate in the function of complexing agent was

added in the first rinsing bath during the enzymatic treatments. It was also added to the PAA bleaching baths where it acted as complexing and buffering agent. The samples were air dried after the treatments.

Evaluation methods

The amount of seed-coat fragments was measured by combing a 100 g of the sample on a Shirley analyser [26]. The outcombed impurities were weighted and their amount was expressed as a percentage of the mass of fibres inserted. Two Shirley tests were made for every sample. The amount of seed-coat fragments was also evaluated visually. Five randomly chosen evaluators evaluated the samples. All the samples with values from 1 to 10, where 1 represents the smallest and 10 the highest amount of seed-coat fragments. (Photos of the samples evaluated with 1 and 10 are shown in Figure 1). An average value for each sample was calculated. The samples were evaluated before and after combing on a Shirley analyser. The difference in valuation before and after combing tells us from which sample the highest amount of impurities was removed mechanically.

Water absorbency was determined according to pharmaceutical prescriptions [25] by measuring the sinking time of 5 g of the sample in distilled water at 20 °C. To fulfil the pharmaceutical prescriptions, the sinking time should be less than 10 seconds. Two parallel measurements were made and averaged.

Fibre damage was assessed in terms of the reduction in the degree of polymerisation (DP) with the use of the viscosimetric method in cuoxam and expressed in Eisenhut's tenderness factors [27].

The residual pectin: equilibrium adsorption of a cationic dye Methylene Blue (MB) from aqueous solution onto cotton

Table 2. Recipes and treatment conditions of different pretreatment processes.

Enzymatic treatment E	PAA bleaching PAA	H ₂ O ₂ bleaching HP	Alkaline scouring A
3 g/l Lawotan RWS 0.05 M acetate buffer 1 g/l Cellusoft L 0.5 g/l Acid Pectinase	15 ml/l Persan S15 46.25 ml/l NaOH 1.5 g/l Na ₄ P ₂ O ₇ 0.3g/l Lawotan RWS	7 g/l H ₂ O ₂ 35% 1 g/l Cottoblanc HTD-N 4 g/l NaOH	3 g/l NaOH 2 g/l Cottoblanc HTD-N
55 °C, 60 min + 80 °C, 10 min	55 °C, 40 min	95 °C, 45 min, hard water	95 °C, 60 min
rinsing: 1x 80 °C, 10min, 1,5g/l Na ₄ P ₂ O ₇ 1x 80 °C, 10min, 2g/l Na ₂ CO ₃ 2x 40 °C, 10min	rinsing: 2x 80 °C, 10min 1x 40 °C, 10min 2x 20 °C, 10min	rinsing: 2x 80 °C, 10 min 2x 40 °C, 10 min	rinsing: 2x 80 °C, 10 min 2x 40 °C, 10 min

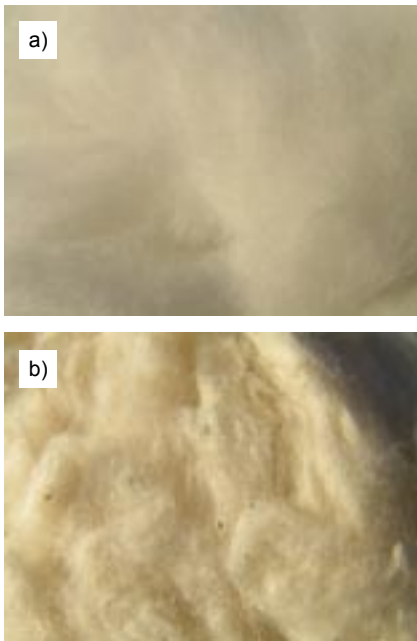


Figure 1. Samples visually evaluated as 1 (a) and 10 (b).

substrate was used to assess how much pectic substance remains after the treatment. This evaluation method is based on the stoichiometric interaction between the dye cation and the carboxylate anion of the pectin. The higher the amount of MB absorbed on the fibres, the higher the content of anionic components is, including pectins [28].

1 g of fibres was dyed with a 0.4 mmol/l MB concentration at a liquor ratio of 1 : 100 for four hours in a shaking bath [29]. Dye uptake of MB was estimated measuring dye concentration with a Cary 1E, Varian UV/VIS double-beam spectrophotometer. From the differences in MB concentration of the dyeing solution before and after dyeing the amount of adsorbed dye was calculated and expressed as a percentage of the initial dye concentration.

The whiteness degree (W) was evaluated by a SF 600+ Datacolor International Spectraflash remission spectrophotometer according to the CIE formula given in EN ISO 105-J02:1999 (light D₆₅, observer 10°). Each sample measurement represents the average of ten readings from different positions on the swatch.

Ash quantity was determined by incinerating 10 g of dry sample at 600 °C for 3 hours, and then weighting it [25]. Two parallel incinerations were made for each sample.

The amount of calcium was obtained by determining the calcium hardness of the water [16b]. The ash obtained from the samples was dissolved in HCl solution. With the addition of 2 N NaOH, the pH was set to 13, and the amount of calcium ions was determined by titration with 0.1 N EDTA solution in the presence of murexid, acting as indicator

Results and discussion

Residual seed-coat fragments are the most resistant impurities of cotton. Our experiments confirmed that neither alkaline nor enzymatic treatment is sufficient to completely remove them, but both processes will make the removal of seed-coat fragments easier in further treatments .

Figure 2 shows the quantity of solid impurities combed out of differently pretreated cotton noil on a Shirley analyser and the subjective evaluation values of raw and pretreated samples before and after combing. Not only seed-coat fragments were removed by combing, but also some entangled short fibres. On the other hand some very small solid particles remained in the material even after repeated combing. Therefore, the results in Figure 2 represent the amount of out-combed solid impurities with adhering tiny fibres. By comparing the results of both properties, the following conclusions were made.

The raw sample had the the smallest out-combed portion, where the highest value was expected, since it contained the high-

est amount of impurities (confirmed by the high subjective evaluation value before combing). This means that particles are strongly anchored into the main body of the fibres and cannot be pulled out by mechanical force. Even mild enzymatic treatment facilitates their removal by combing. It can be seen that the amount of outcombed particles was higher in the samples treated with cellulases (Ec and Ecp). We can conclude that the cellulase treatment facilitated the mechanical removal of seed-coat fragments.

This pattern is repeated for samples that were further bleached with PAA or HP. More seed-coats were removed from samples which were treated with cellulases (Ec+PAA, Ecp+PAA, Ec+HP, Ecp+HP). The amount of particles removed was much higher for PAA bleached samples. This can be explained with subjective evaluation, which shows that a lot of seed coat fragments remained on the fibres after enzymatic treatment and PAA bleaching. Both processes proceed at the low temperature of 55 °C and mild pH of 5. These conditions were too mild to swell or to degrade the seed coat fragments. Only their mechanical removal was easier. On the other hand, the HP degraded the majority of seed-coat fragments. Very low subjective evaluation values show that there were no visible seed-coat fragments on HP bleached samples. They were either white or destroyed and consequently the outcombed quantity of impurities was smaller.

In the alkaline scoured sample (A) the amount of out combed particles was

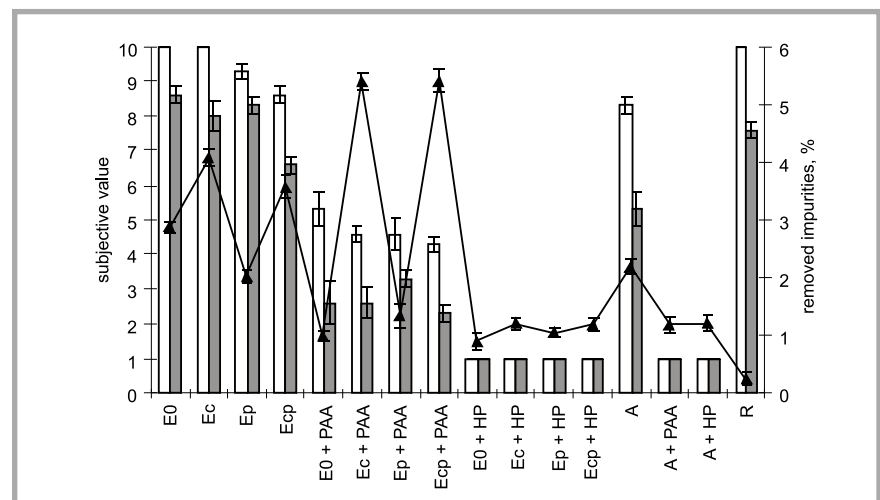


Figure 2. Subjective evaluation values of raw and pretreated samples before \square and after \blacksquare combing and the percentage of impurities - \blacktriangle - removed from different samples on Shirley analyser; the straight line segments which joins the measuring points visualises do not present any function.

smaller than in the enzymatically treated samples - Ec and Ecp. The visual evaluation value before combing for A was better than for Ec and Ecp and improved markedly after combing. This means that the seed-coat fragments were partly degraded during alkaline scouring, their amount on fibres decreased and many of the remaining seed-coat fragments were easily combed out. Samples that were bleached after alkaline scouring (A+PAA and A+HP) were visually evaluated as clean, without visible seed-coat fragments and, as expected, the amount of out-combed particles was low.

Closer review of Figure 2 reveals that the samples R, E0 and Ec were evaluated as the most impure samples. This means

that treatment with cellulases alone did not remove the seed-coat fragments. They were removed only in the mechanical aftertreatment, as shows the improved value for Ec after combing. Slightly better values were achieved by pectinase treated samples (Ep), and somewhat better by pectinase/cellulase treated samples (Ecp). We can conclude that pectinases enabled the removal of some impurities during the treatment but they did not accelerate their removal in combing. We can also conclude that pectinases and cellulases have a synergistic effect on the removal of seed-coat fragments. Pectinases hydrolyze the pectic substances in the primary wall of the fibres and facilitate the cellulases in hydrolysing the tiny fibres attached to the seed husk.

Table 3. Ash and calcium content, sinking time, *t* and the amount of absorbed methylene blue dye, MB.

procedure	ash ^a , %	Ca, ppm	t ^a , s	MB, %
E0	0,47 (0,023)	497	122 (3,5)	85,35 (8,1)
Ec	0,36 (0,031)	558	54 (6,2)	80,47 (6,2)
Ep	0,34 (0,086)	451	65 (5,2)	84,01 (7,3)
Ecp	0,33 (0,047)	422	15 (0,0)	78,80 (6,3)
E0+PAA	0,27 (0,024)	448	82 (10,5)	91,60 (7,9)
Ec+PAA	0,24 (0,047)	417	77 (4,0)	88,62 (9,1)
Ep+PAA	0,20 (0,021)	496	38 (4,0)	86,61 (8,4)
Ecp+PAA	0,16 (0,028)	321	15 (0,7)	85,40 (6,2)
E0+HP	0,63 (0,026)	1192	5 (0,2)	49,82 (3,3)
Ec+HP	0,56 (0,021)	1131	4 (0,6)	45,52 (5,8)
Ep+HP	0,54 (0,038)	1434	4 (0,2)	49,32 (9,2)
Ecp+HP	0,52 (0,028)	1389	4 (0,0)	44,07 (9,8)
A	0,63 (0,036)	691	32 (2,2)	79,48 (7,6)
A+PAA	0,22 (0,029)	318	7 (0,2)	66,62 (7,5)
A+HP	0,69 (0,047)	1099	2 (0,5)	56,11 (9,4)
R	1,21 (0,033)	1024	∞	21,26 (10,2)

Table 5. Whiteness degree (CIE *W*), lightness (*L*^{*}), chroma (*C*^{*}), hue (*h*) and tint value (*TV*).

procedure	CIE <i>W</i>	<i>L</i> [*]	<i>C</i> [*]	<i>h</i>	<i>TV</i>
E0	-0,3	81,25	11,69	80,01	-9,0
Ec	3,0	81,92	11,30	81,14	-8,2
Ep	2,9	82,22	11,49	80,85	-8,4
Ecp	4,3	82,51	11,32	81,34	-8,1
E0+PAA	50,0	90,88	5,99	93,89	-1,6
Ec+PAA	49,5	90,59	5,95	93,52	-1,7
Ep+PAA	51,5	90,81	5,65	94,02	-1,5
Ecp+PAA	52,8	991,98	5,97	94,24	-1,5
E0+HP	78,5	91,29	0,24	143,16	0,3
Ec+HP	79,3	92,17	0,45	123,24	0,3
Ep+HP	80,3	92,97	0,61	112,08	0,2
Ecp+HP	82,3	93,47	0,43	121,52	0,2
A	15,2	83,98	9,89	79,97	-7,4
A+PAA	63,1	91,81	3,70	95,67	-0,8
A+HP	76,1	92,49	1,27	105,10	0,1
R	6,5	83,99	11,53	84,25	-7,0

Table 4. Average polymerisation degree (DP) and Eisenhut's tendering factor (*s*) of selected samples; ^aStandard deviations in brackets.

Procedure	DP ^a	<i>s</i>
E0	2754 (19)	
Ecp	2778 (3)	-0,009
Ecp+PAA	2577 (15,5)	0,070
Ecp+HP	1851 (14,5)	0,437
A	2660 (12)	0,036
A+PAA	2569 (3,5)	0,069
A+HP	1906 (0,5)	0,340

The amounts of ash and calcium, the absorption of Methylene Blue dye and the sinking times are presented in Table 3. The lowest amounts of ash and calcium and the lowest absorption of MB within each group of samples had those, which were treated with a combination of cellulases and pectinases. These samples also had the shortest sinking times. These results confirm the synergistic effect of both enzymes in the removal of pectin and other noncellulosic components from the fibres.

The HP bleached samples had higher ash and calcium content because of the hard water used in the bleaching process. Bleaching with HP in hard water is a usual practice since the magnesium ions stabilise the HP solutions [16]. However, all the HP bleached samples absorbed less MB dye and had shorter sinking times than PAA bleached or only scoured samples. The alkaline scoured and PAA bleached samples (A+PAA) had similar results. All these samples were treated at least once in hot alkaline solution, which enabled the removal of noncellulosic in-crusts to the required level. The samples that were treated only at mild enzymatic and PAA conditions retained a higher amount of pectins and waxes, and therefore remained less hydrophilic.

Raw, untreated fibres are very hydrophobic- they did not sink in water, and therefore their MB absorption was very low.

The average polymerisation degree (DP) is a measure of fibre damage and is presented in Table 4. No decrease in DP was observed after enzymatic scouring and only a small decrease after alkaline scouring. PAA bleaching also caused only a small decrease in DP, whereas a remarkable decrease was measured after HP bleaching. From the values of Eisenhut's tendering factor, it can be concluded that fibres are not damaged

in processes which include enzymes and/or PAA.

Table 5 represents the CIE whiteness degree (W), lightness (L*), chroma (C*), hue (h) and tint value (TV). Enzymatically treated samples had a very low whiteness degree, even lower than raw fibres. Alkaline scouring increased the whiteness degree by about ten units. Bleaching with PAA resulted in poor whiteness improvement. The difference of ten units between enzymatically and alkaline scoured samples remained after PAA bleaching. The highest whiteness degrees had samples bleached with HP. Bleaching with HP equalised the initial difference between enzymatic and alkaline scouring. It can also be observed that within a particular group of samples, those treated with pectinases and cellulases had the highest whiteness degree.

All samples, except those bleached with HP, had red hue (negative TV). Whiteness and other measured properties indicate that HP bleaching removes more impurities from the fibre surface than PAA bleaching. Because PAA is a stronger oxidising agent than HP, the lower effects of cleaning are a consequence of milder bleaching conditions. At least one treatment at high pH and high temperature is necessary for efficient removal of seed-coat fragments and other impurities from fibres.

Conclusions

- The combined treatment with cellulases and pectinases has a beneficial effect on the removal of seed-coat fragments from cotton fibres. Cellulases hydrolyse some tiny fibres that attach the seed husk to the moiety of the fibres, and consequently they can be easily combed out. Pectinases hydrolyse the pectic substances in the primary wall of the fibre and facilitate the approach of cellulases to cellulose. Pectinases also act on the pectic substances in the anchoring area of the fibre's foot and facilitate the separation of fibre bases from the seed-coat.
- The PAA bleaching improved the purity of enzymatic scoured fibres, but water absorbency and the whiteness value were still insufficient. Seed-coat fragments remained visible after PAA bleaching.
- HP bleaching after enzymatic treatment removed most of the non-

cellulosic impurities and improved whiteness and water absorbency to a desired level.

- For effective removal of seed-coat fragments, the following procedures are suitable:
 - treatment with a combination of cellulases and pectinases, followed by hydrogen peroxide bleaching or
 - alkaline scouring, followed by either hydrogen peroxide or peracetic acid bleaching.
- Insertion of enzymatic scouring or PAA bleaching into the pretreatment of cotton results in very low fibre damage and an environmentally friendlier process with lower consumption of energy and water.

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