Enhancing the accessibility of starch size and cellulose to enzymes in raw cotton woven fabric by air-plasma pretreatment

Abstract

In this paper raw cotton fabric was pre-treated with non-thermal atmospheric air-plasma and the accessibility of the surface polymers of the fibres and yarns that act as respective substrates for the enzymes was evaluated. Results proved that plasma slightly destroyed and oxidized the starch size on the surface of warp yarns and partially removed the thin and perfectly hydrophobic waxy coverage of the fibres in weft yarns, creating deep "pits" with a depth of 215 nm. This latter process contributed to the exposure of cellulose and pectin located under the waxy outer layer of the elementary fibres in the weft yarns, and significantly increased the surface roughness of the fibres (from R_q 25 to 67 nm, for the raw and 180 s plasma treated samples, respectively). Amount of the reducing sugars released during the amylase and cellulase digestion of the plasma treated fabrics confirmed that air-plasma significantly increased the accessibility of the starch and cellulose, respectively, to the enzymes and resulted in an enhanced solubilisation of both polymers. Since the plasma-treated substrates displayed significantly faster enzyme reactions, the time of enzymatic treatments can be shortened sharply.

Keywords: Enzyme, Accessibility, Starch, Cellulose, Pectin, Desizing

Introduction

Recently, a number of shorter studies and comprehensive reviews have been published regarding the effects of plasma treatment on the surface properties of natural and synthetic fibres, yarns and fabrics. By using non-thermal plasma treatment, the surface layers of the fibrous substrates can be activated and modified without alteration of the bulk characteristics. Atomic temperatures of the non-thermal atmospheric plasma are close to ambient, but electron temperatures reaching values up to orders of magnitude higher. Thus, plasma with such characteristics readily interacts with solid surfaces, causing reactions that would otherwise occur only at elevated temperature.[1-4]

In a raw cotton woven fabric, the warp yarns are covered by mostly a starch containing sizing agent to improve the weaving efficiency. The weft yarns, however, do not contain any size and their surface characteristics are determined solely by the constituents (such as waxes, cellulose, pectins, etc.) located in the outer surface layers of the cotton elementary fibres. Both the starch sizing agent and the non-cellulosic constituents of the fibres have to be completely removed in order to achieve a perfectly hydrophilic fabric with an appropriate whiteness and without any impurities. In the environmentally-friendly enzyme-aided textile technologies amylase enzymes are widely used for the degradation and removal of the starch containing sizing agent. [5-7] For accelerating the removal of the waxy outermost layer of the cotton elementary fibres and the natural colouring matters, pectinase and glucose oxidase enzymes can be applied, respectively. Cellulase enzymes are usually used in the biofinishing of cellulosic textiles. [8-14]

Plasma pretreatment can affect the removal of both sizing agent and waxy surface layer. Cold-plasma in oxygen[15], oxygen/helium[16,17] and air[18] atmosphere was highly effective in removing the starch size from the surface of woven fabrics, resulting in a significant increase in surface hydrophilicity. The maximum weight loss values measured in

oxygen/helium and air plasma were 2.92 %[16] and 6 %[18], respectively. The surface roughness of the size layer characterized by the root mean square roughness (rms) increased significantly (from 2.8 to 33.1 nm) with increasing the time of oxygen/helium plasma treatment (from 0 to 45 s, respectively).[17] Furthermore, plasma pretreatment accelerated also the degradation of the residual starch size in the subsequently applied desizing process with $NaHCO_3$.[17] Plasma was proved to be effective also in degradation of the waxy outer layer of cotton fibers.[19] By the effective removal of the waxy materials from the fibre surface with a short plasma pretreatment, the processing time of the subsequently applied conventional chemical processes was reduced significantly.[20]

Furthermore, plasma pretreatment can increase the efficiency of the enzyme-aided textiles processes by accelerating the enzyme action. The efficiency of pectinase enzymes in bioscouring of cotton and linen was enhanced by different plasmas (i.e. at atmospheric and low pressure, in air/argon/oxygen gases).[21-24] The synergetic effect of plasma and cellulase enzyme treatments on the physical and chemical properties of cotton fabric was also proved.[25] In spite of that there are a lot of benefits obtained by using plasma as a pretreatment in the enzyme-aided processes, the effects of plasma on the efficiency of the subsequently applied enzyme catalyzed reactions have not yet been fully evaluated and discussed. Furthermore, nothing is known about the accessibility of the surface polymers of the fibres and yarns that act as respective substrates for the enzymes.

In this paper, we focused on how the air-plasma treatment changed the raw cotton fabric surface and how these changes improved the accessibility of polymers located on the surface of warp yarns (i.e. starch) or under the surface layers of cotton fibres (i.e. cellulose) to an amylase and cellulase enzyme, respectively. Exposure of pectin by plasma treatment was also discussed.

Experimental

Fabrics and enzymes

Raw cotton fabric, plain-weave, with a fabric weight per unit area of 153 g/m^2 and a fabric count (warp/weft) of 28/24 yarn/cm was selected for the experiments. Warp yarns of the fabric were sized by a standard starch-based sizing material. The fabric was cut to the required dimension (10 cm × 20 cm, approximately 6 g) and these samples were then conditioned at 65 % relative humidity and 20 °C for 24 hours prior to the plasma treatment and any of the testing. Bleached cotton fabric (scoured with sodium hydroxide and bleached with hydrogen peroxide) with a weight per unit area of 150 g/m^2, plain-weave, was selected as a perfectly hydrophilic and accessible substrate with a cellulose content of nearly 100 % and used exclusively in the cellulase enzyme hydrolysis without any pretreatment. Both fabrics were supplied by Pannon-Flax Linen Weaving Co., Hungary.

Two commercial enzymes, an amylase and a cellulase were used for characterizing the accessibility of the plasma treated fibre surface to enzymes. A commercial α-amylase (Beisol LZV) recommended for desizing of woven fabrics with starch-based sizing agent was kindly supplied by CHT Bezema AG (Switzerland) and used as received. The enzyme reaches the optimum effectiveness in the pH and temperature range between 5.4 – 8.0 and 65 - 75 °C, respectively. Celluclast 1.5L, a commercial cellulase enzyme mixture produced by *Trichoderma reesei* was received from Sigma-Aldrich. The enzyme exhibits maximal activity near 50 °C and at pH 4.5-5.0. All other chemicals used in this work were reagent grade.

Plasma treatment

Non-thermal plasma treatment was performed in ambient air, by a diffuse coplanar surface barrier discharge (DCSBD) type equipment (Manufacturer: Roplass s.r.o., Brno,

Czech Republic). The plasma is ignited with sinusoidal high frequency, ~10-20 kHz, high voltage with peak-to-peak values of up to 20 kV. Both sides of the cotton fabric samples were treated, applying a power of 300 W and treatment times of 30, 90 and 180 s.

Characterization of the accessibility of polymers in the fibre surface to enzymes

Surface accessibility of the raw cotton fabric to the amylase and cellulase enzymes before and after the plasma treatment was evaluated by measuring the reducing sugars liberated during the enzymatic hydrolysis of starch size and cotton cellulose, respectively. In the cellulase experiments, enzymatic hydrolysis of a bleached cotton fabric was also tested only for comparison.

The amylase enzyme was diluted with tap water to 500 fold. The untreated and plasma treated cotton fabric samples of 0.4 g were treated with the diluted enzyme solution containing in a shaking waterbath (Medingen SWB 20) at 70 °C for 210 min with a shaking frequency of 120 rpm. The fabric to liquor ratio was 1:50. The cellulase enzyme was diluted in 0.05 M acetic acid/sodium acetate buffer (pH 5) to 250 fold. 0.6 g of each of the cotton samples were put into an Erlenmeyer flask which was filled with the pre-warmed diluted enzyme solution. The fabric to liquor ratio was 1:100. The flask was placed in a shaking waterbath controlled to 50 °C and with a shaking frequency of 120 rpm for 60 min.

Dinitrosalicylic acid (DNS) colour test for determining the reducing sugars liberated by the enzymes was used. The 3,5-dinitrosalicylic acid is reduced to 3-amino-5-nitrosalicylic acid, while the aldehyde groups appear to be oxidized to carboxyl groups. Absorbance of the coloured reaction products can be measured at 540 nm. Concentration of the reducing sugars was determined as follows: 0.5 ml of enzyme sample solution was diluted with 1 ml of acetate buffer at pH 5, and the enzyme reaction was terminated by the addition of 3 ml DNS solution, followed by boiling for 5 min. After cooling, the absorbance was read at 540 nm. The

liberated reducing sugars in glucose equivalent were estimated according to Miller.[26] Each reported value is the average of at least three parallel measurements.

It has to be noted that an attempt to characterized the accessibility of pectin by the action of a pectinase enzyme was also made, but because of the very low pectin content of cotton fibres, it was impossible to follow the enzyme catalysed hydrolysis of pectin even after plasma treatment by measuring the reducing sugars as described in the previous paragraphs.

Analysis of the surface and bulk properties

Contact angle measurements were carried out at 22 ºC and 55 % relative humidity using a Ramé-Hart goniometer (USA) with a drop image standard software of DT-Acquire and a camera. Liquid drops of 20 µl distilled water were deposited on each fabric sample and the image of the drops deposited onto the fabric surface was captured immediately by the camera. The value reported is the average of at least 5 readings for each of the samples. Wettability of the plasma treated cotton fabrics was characterized by water drop test, counting the elapsed seconds between the contact of the water drop with the fabric and the disappearance of the drop into the fabric. A burette was used to drop a single drop of water on the sample from a height of 1 cm. Ten readings were taken from different locations on the samples subsequent to the plasma treatment and the average was calculated.

X-ray photoelectron spectroscopy (XPS) was done by a Kratos XSAM 800 spectrometer using Mg $K\alpha_{1,2}$ radiation and fixed analyser transmission mode (80 and 40 eV pass energies for survey and detailed spectra, respectively). The diameter of the analysed spot was about 2 mm. The spectra were referenced to the C 1s line (binding energy, BE=285.0 eV) of the hydrocarbon type carbon. Data acquisition and processing were performed with the Kratos Vision 2 program**.** The conditions of the measurements and the spectrum elaboration

149 were kept constant, and thus the intensities measured on parallel samples were repeatable with
150 good precision (within ± 5 %).

151 Some of the samples were selected for characterizing the surface morphology by a DI
152 Nanoscope Dimension 3100 AFM (Digital Instruments Inc., USA, Santa Barbara). The fibres
153 from weft yarns of the untreated (raw) and plasma treated (for 180 s) fabrics were bent on a
154 flat sample holder and fixed by glue. A series of atomic force microscopy (AFM) images in
155 the scan size range 2-20 mm were acquired. These images are proper 3D profiles of the
156 sample surface and can be represented in a so called 3D view, where an axonometric coloured
157 picture is calculated from the measured spatial data representing a microscopic, but
158 perspective view of the surface. The rms roughness (the root mean square of height deviations
159 compared to the average plane of the image in each pixel) was calculated for surface
160 topography quantification and presented as R_q values for both samples.

161 Infrared spectroscopy (ATR FT-IR) measurements of the cotton fabrics were carried
162 out applying a Tensor 27 spectrophotometer (Bruker, Germany) with a diamond ATR cell
163 (Bruker Platinum ATR) in the wavenumber range of 4000-400 cm^{-1} using 2 cm^{-1} resolution
164 and 32 scans. The relative peak intensities were also calculated in comparison to 609 cm^{-1},
165 which can be assigned to the OH-out of plane bending in cellulose and hence it does not vary
166 with the plasma treatment.[27,28]

167 Efficiency of the amylase in degradation and removal of starch sizing agent was
168 characterized by the Tegewa scale method using an iodine/potassium iodide.[29] One drop of
169 the solution was put on the fabric after amylase treatment and gently rubbed. The starch size
170 residue was determined visually by comparing the stained fabric swatch to the Tegewa violet
171 scale, where 1 is dark blue and 9 has no colour stain.

172 The effect of plasma treatment on the tensile properties of raw cotton fabric was
173 determined by ravelled strip test (1.5 cm width) on an Instron Tester Model 5566 (USA,

ASTM 1682). Breaking load and elongation at rupture of the raw and plasma treated fabrics were measured in both warp and weft directions.

Results and discussion

Effect of plasma on the surface and bulk properties of the fabric

Before discussing the effects of plasma treatment on the accessibility of enzymes to the polymers of the surface of raw cotton fabric, it has to be underlined that the warp yarns are covered with a starch-based and lubricant containing sizing agent. The thickness of a starch size film on the surface of cotton warp yarns, sized with low pressure or high pressure squeezing, was proved to be 14 and 8 μm, and the thickness of the penetration layer under the size film was 8 and 20 μm, respectively.[30] The weft yarns, however, do not contain any starch size, they consist only of elementary cotton fibres with a hydrophobic waxy outer layer called cuticle, which can be characterized by a thickness of about 12 nm.[31] Cuticle is composed mainly of non-cellulosic lipophilic components such as waxes, fats and resins, and also pectin and less ordered cellulose.[8]

AFM images of the fibres derived from the weft yarns of untreated and plasma treated (for 180 s) fabrics in Figure 1 show that plasma created deep "pits" with a depth of 215 nm. Significantly larger pits with depths of 1000-2000 nm were created on the surface of bleached flax fibres by oxygen plasma.[32] Compared to the surface roughness of the untreated raw fibre (Figure 1, left; R_q 25 nm), the plasma treated (Figure 1, right) fibre surface has a significantly rougher structure with an R_q value of 67 nm. We assume that the applied plasma treatment can only etch and attenuate the relatively thick (i.e. 8-20 μm) sizing layer on the warp yarns, but it can contribute to the localized ablation of the waxy outer layer of the elementary fibres in the weft yarns, making the fibre surface highly rough.

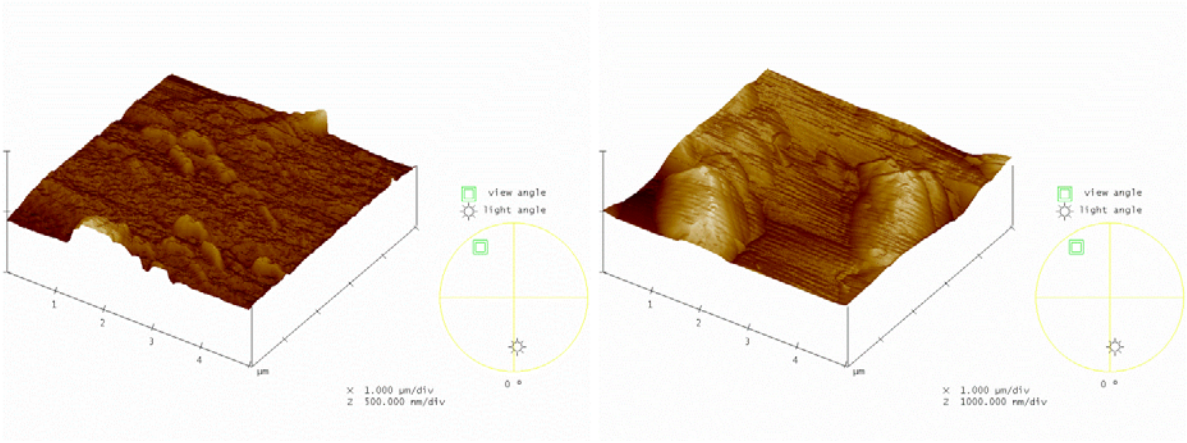


Figure 1. AFM images of the elementary fibres of weft yarns derived from raw (left) and air-plasma treated cotton with exposure time of 180 s (right). Image scale: 5 µm in *x* and *y* directions and 500 nm in *z* direction.

Changes in the waxy surface layer of the elementary fibres in the weft yarns were also proved by ATR FT-IR (Figure 2). Since the penetration depth of light is typically of the order of a few microns (ca. 0.5-3 µm), the results provided by FT-IR ATR were used successfully for characterization of the plasma-induced changes in constituents of the surface of elementary fibres in weft yarns. Extra peaks in the spectrum of the raw fabric at 2916 and 2850 cm^{-1}, associated with the asymmetric and the symmetric stretching of methylene groups in long alkyl chains, indicate the presence of waxy materials in the fibre surface.[33] For plasma treated samples, however, the decrease in relative intensities of the peak at 2850 cm^{-1} (Table 1) and the absence of the peak at 2916 cm^{-1} (Figure 2) was observed, indicating a partial removal of the waxy outer layer of the elementary fibres in weft yarns by plasma treatment.

For characterizing the chemical changes of the fabric surface occurred by plasma treatment, XPS was used and a spot with a diameter of about 2 mm and with a sampling depth of 10 nm was analysed. The samples selected for this study included the untreated raw cotton and the plasma treated fabrics with exposure times of 90 and 180 s. The O/C ratio found for raw cotton fabric was 0.25 (Table 1), which was higher than that expected for a purely waxy

surface (O/C ≈ 0.11)[34,35] and lower than that measured for the starch containing sizing agent (O/C ≈ 0.34)[17]. A comparison of the O/C values suggested that the surfaces analyzed by XPS were composed of the starch and lubricant containing sizing agent on the warp yarn, and of the waxy cuticle of the elementary fibres in the weft yarns. They are consistent with the high percentage of carbon in C-C and C-H type (76 %), as well as in C-O type (18 %) bonds in the surface layers.

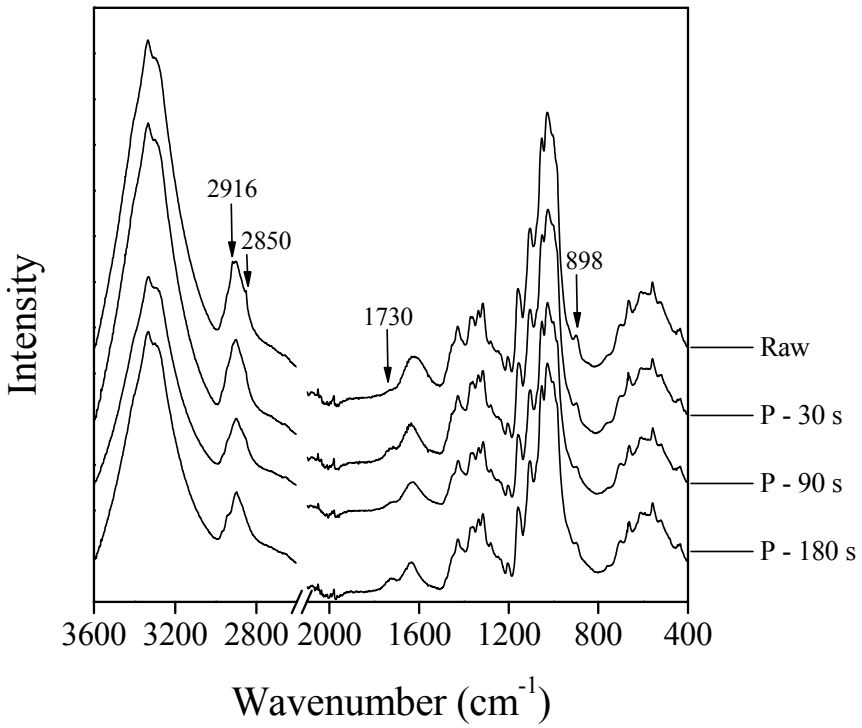


Figure 2. ATR FT-IR spectra of the raw and air-plasma treated (P) cotton fabrics with exposure time of 30 s, 90 s and 180 s.

As seen in Table 1, the O/C ratios for the plasma treated samples were significantly higher (for 90 s: 0.54; for 180 s: 0.82) than for that of the raw cotton (0.25). Upon plasma treatment, both components C2 (286.7 eV, carbon in C-OH and C-O-C) and C3 (288.3 eV, carbon in C=O and O-C-O) increased (Figure 3 and Table 1). It is remarkable that the surface O/C atomic ratio of the fabric treated with plasma for 180 s is 0.82, which was almost equal to

234 0.83, which was calculated to the pure cellulose[36]. The increase of the surface concentration
235 of C atoms bonded to oxygen was compensated mainly by the decrease of the C1 component
236 (285.0 eV, pertaining to carbon C-C and C-H bonds). The significant increase of the O/C ratio
237 and of the concentrations of the various oxidized states of carbon (mainly C-O, but also C=O
238 and/or O-C-O) suggests the introduction of some hydrophilic groups to the surfaces by
239 plasma-aided bond scission and oxidation of the starch molecules in the sizing agent, and the
240 exposure of cellulose and other polymers (such as pectin, O/C ratio: 0.94; unpublished data)
241 to the surface of elementary fibres in the weft yarns by the partial degradation of the waxy
242 materials upon plasma treatment. Furthermore, the oxidation of the latter polymers (i.e.
243 cellulose and pectin) can also occur. An increase in O/C ratio was also detected in the low-
244 pressure oxygen plasma treatment of cellophane foils, which was attributed to the
245 decomposition of polymer chains and the oxidation reactions[37]. While the increase in
246 aldehyde groups in the sample treated with plasma for 480 s indicated a partial decomposition
247 of the cellulose macromolecules, the increased number of carboxylic and/or carboxylate
248 groups revealed oxidation reactions of the C-OH groups.
249
250

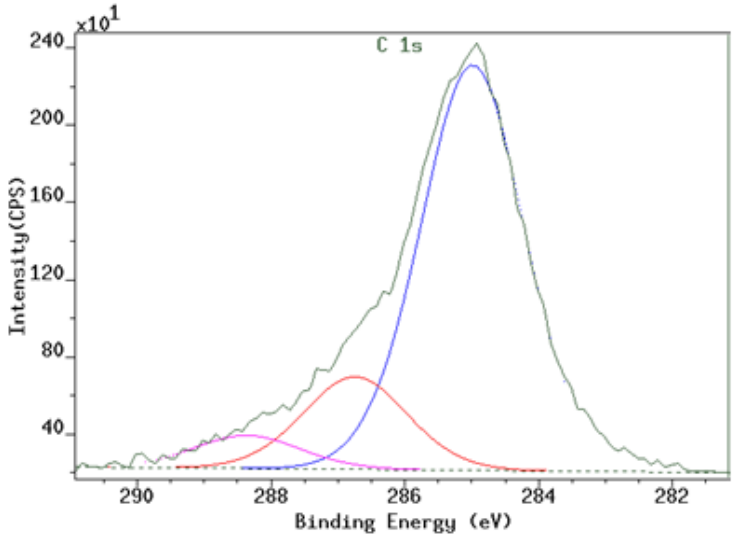
Table 1. Relative intensities for IR absorption bands, O/C atomic ratio and results of deconvolution of the C 1s peak by XPS for the untreated (raw) and air-plasma treated cotton fabrics as a function of time of plasma treatment.

	Relative intensities at[a]				C1 %	C2 %	C3 %
Fabric samples	2850	1730 (cm^{-1})	898	O/C	285.0 eV C–C, C-H	286.7 eV C–O	288.3 eV C=O, O–C–O
Raw	1.01	0.25	0.67	0.25	76	18	6
Plasma treated – 30 s	0.97	0.31	0.70	n.d.[b]	n.d.	n.d.	n.d.
Plasma treated - 90 s	0.98	0.37	0.72	0.54	57	25	18
Plasma treated - 180 s	0.95	0.41	0.73	0.82	45	38	17

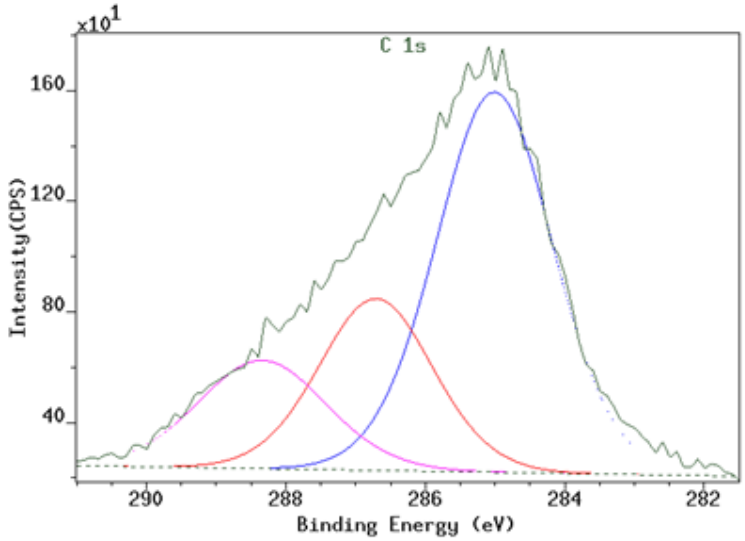
[a] compared to 609 cm^{-1}

[b] n.d. - not determined

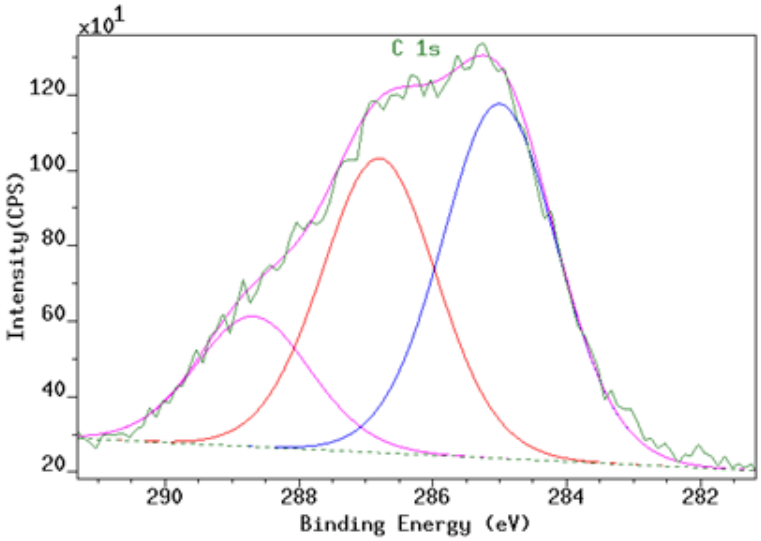
264



265



266



267

Figure 3. Deconvolution of the C 1s peak. Untreated raw cotton (top); air-plasma treated cotton with exposure time of 90 s (middle) and 180 s (bottom).

Exposure of cellulose and pectin in the elementary fibres of weft yarns was confirmed by ATR FT-IR (Figure 2). Significant changes were observed in the ester region at ~1730 cm^{-1} as well as at 898 cm^{-1}. The former is related to the pectin molecule, since approximately 70 % of carboxyl groups of galacturonic acid units in pectin are esterified with a methoxyl group, and the latter is associated with the C_1-O-C_4 β-glycosidic bonds present in cellulose molecule.[8,25] Results in Table 1 proved that the relative intensities increased significantly by plasma treatments (at 1730 cm^{-1}: 0.25 versus 0.31, 0.37 and 0.41; at 898 cm^{-1}: 0.67 versus 0.70, 0.72, 0.73; for raw versus plasma treated fabrics with exposure time of 30 s, 90 s and 180 s, respectively). This means that the partial degradation and removal of the waxy outer layer of cotton, which occurred during the applied air-plasma treatment, exposed the pectin and cellulose constituents and made them more detectable on the fibre surface by ATR FT-IR.

Both degradation of the waxy outer layer of the elementary fibres and introduction of some hydrophilic groups to the surfaces significantly modified the absorbency of raw cotton fabric and provided a hydrophilic fabric surface, which can be characterized by a short wetting time (a few seconds only) and a low water contact angle (less than 30 °), when the plasma treatment was carried out for 180 s (Table 2).

Table 2. Values of wetting time, water contact angle and tensile properties of the untreated (raw) and air-plasma treated cotton fabrics as a function of time of plasma treatment.

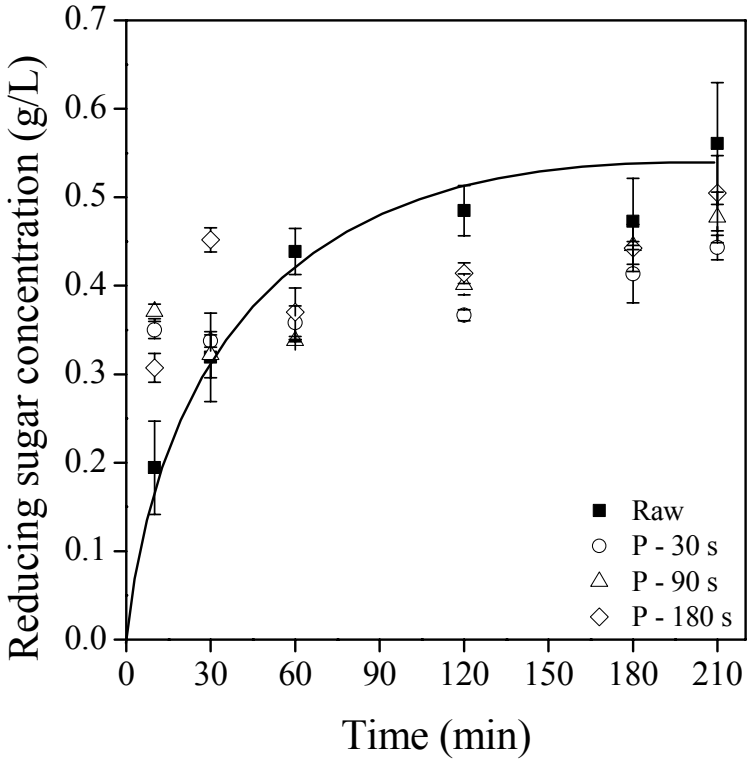
Fabric samples	Water wetting time (s)	Water contact angle (°)	Breaking load (N)		Elongation (%)	
			Warp	Weft	Warp	Weft
Raw	> 180	106 ± 6	113 ± 7	105 ± 12	14.5 ± 0.3	14.7 ± 0.7
Plasma treated – 30 s	143 ± 19	82 ± 4	111 ± 11	111 ± 10	13.4 ± 0.4	15.1 ± 0.9
Plasma treated - 90 s	17 ± 7	61 ± 3	111 ± 5	112 ± 4	13.9 ± 0.4	14.3 ± 0.5
Plasma treated - 180 s	5 ± 4	29 ± 6	116 ± 10	117 ± 11	13.8 ± 0.9	13.9 ± 0.5

Since these changes in the surface, to an extent, could affect tensile properties of the fabrics, an investigation was conducted to determine the breaking load and elongation of the fabrics. Since any shrinkage and alteration in appearance of the fabrics did not occur during the DCSBD plasma treatment (namely, there was no change at all in the fabric count by plasma), the dimensional changes did not bias the data derived from the tensile testing. Thus, it can be concluded from the results in Table 2, that the applied plasma treatments did not cause significant change in tensile properties of the raw cotton fabric. None of the data in Table 2 reveals fabric degradation.

Enzymatic hydrolysis of starch size on the yarn surface

An amylase enzyme was chosen for characterizing quantitatively first, the effect of plasma on the hydrolysis rate of starch size, second, the changes in the amount of starch occurred by plasma treatment and third, the efficiency of the enzymatic desizing process. For raw and plasma treated cotton fabrics, Figure 4 shows the concentration of reducing sugars liberated from the starch size as a function of time of enzyme hydrolysis.

It is obvious that in the first 10 minutes the amylase was much more efficient in solubilization of starch from the plasma treated fabrics (closed symbols) than from the untreated one (■). We remember that hydrophilicity and surface roughness of the plasma treated cotton fabrics is significantly higher (Table 2 and Figure 1, respectively) than those of the raw fabric, which are presumably the main reasons of the increased and fast solubilization from the plasma treated fabrics. Starch in raw cotton, however, had a lower accessibility to amylase, which could be explained by the compact and hydrophobic surface of warp yarns.



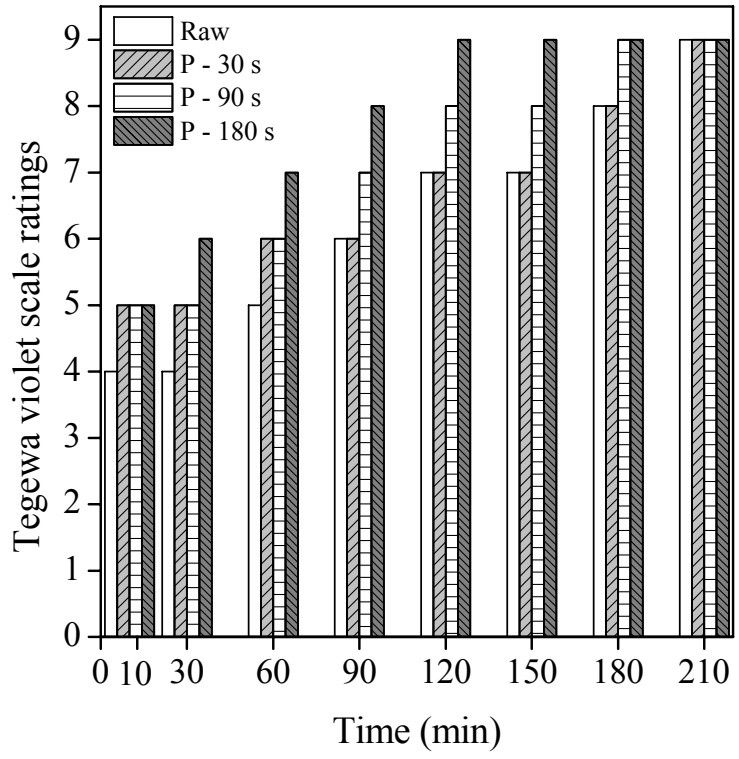
326

327 **Figure 4.** Reducing sugars released from the starch size in raw (filled squares, solid line) and
328 air-plasma (P) treated (for 30, 90 and 180 s) cotton fabrics (closed symbols) by 10 % of
329 amylase enzyme as a function of time of hydrolysis.

330

331 Results also reveal that concentration of reducing sugars released from the plasma
332 treated fabrics increased rapidly at first, then levelled off after 60 min in the range of 0.34 -
333 0.47 g/l. It is noteworthy that almost the same level of reducing sugar concentrations was
334 reached from the plasma treated fabrics and the values indicated only marginal differences
335 among the fabrics treated with plasma for different time. For raw cotton, however, the
336 concentration of reducing sugars increased gradually and levels off after about 120 min at
337 about 0.51 g/l. Thus, the maximal amount of reducing sugars released from the plasma treated
338 fabrics was slightly lower than that from the raw cotton.

339 Furthermore, the iodine solution test (Tegewa) proved that amylase treatment for 210
340 min removed perfectly the total amount of starch from all fabrics (Figure 5). Since the
341 maximum reducing sugar data (in Figure 4) are directly proportional to the amount of starch
342 on the fabrics, it is obvious that plasma treatment slightly degraded the starch sizing agent on
343 the surface of warp yarns. Notwithstanding that we could not detect any considerable change
344 in the weigh per unit area of the raw fabric upon plasma treatment, the results from amylase
345 hydrolysis proved a partial (10-20 %) removal of starch by plasma etching.
346



347

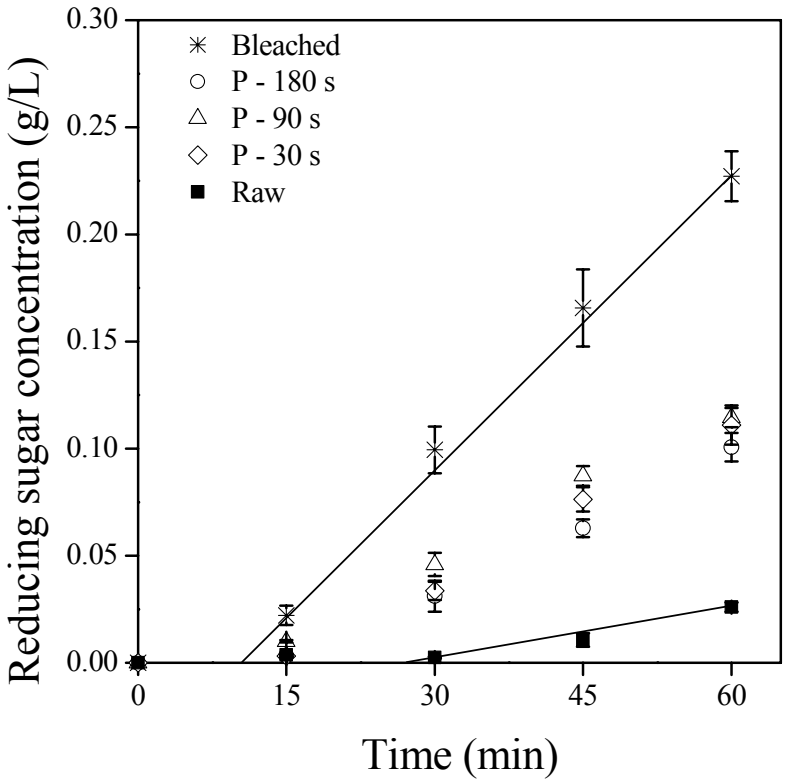
348 **Figure 5.** The Tegewa violet scale ratings for raw and air-plasma treated (P) fabrics (for 30,
349 90 and 180 s) as a function of time of amylase treatment.

350

351 Highly efficient hydrolysis of the lower amount of starch on the plasma treated fabrics
352 resulted in an increased desizing effect, which can lead to a significant shortening of the
353 desizing process. The plasma treated fabrics showed the commercially acceptable violet scale

354 rating of 5 with a hydrolysis time of 10 minutes. For the untreated fabric, however, a longer
355 enzyme treatment (60 minutes) was required to obtain acceptable desizing effect (Figure 5).
356 Thus, the increase hydrophilicity and accessibility of the plasma etched surface and the more
357 efficient hydrolysis of the residual starch resulted in an increased desizing effect, which can
358 lead to a significant shortening of the desizing process (from 60 to 10 minutes).
359 *Enzymatic hydrolysis of cellulose in the fibre surface*
360 Plasma-aided degradation and removal of the waxy cuticle of the cotton fibres in the
361 weft yarns can contribute to the exposure of cellulose. For detecting the presence of cellulose
362 and characterizing its accessibility, a cellulase enzyme was used as an analytical tool and the
363 amount of reducing sugars released from the raw and plasma treated cotton fabrics was
364 measured and compared to that released from the bleached cotton. It can be considered as a
365 highly accessible and pure cellulose sample.
366 In the plot of Figure 6 the concentration of reducing sugars released from different
367 fabrics is shown as a function of time of cellulase hydrolysis. In raw cotton, the elementary
368 fibres in the weft yarns are perfectly hydrophobic, because the waxy materials evenly cover
369 the cotton fibre, hindering the hydrolysis of cellulose by a cellulase enzyme. More than 27
370 minutes (intersection of line on abscissa) were needed for releasing reducing sugars in
371 detectable amount. Due to the fairly intact structure, cotton fibres in raw cotton fabric have
372 the lowest accessibility to the cellulase enzyme, since the waxy materials prevent the access
373 of enzyme to cellulose.
374 A dramatic increase in the rate of cellulose hydrolysis in the plasma treated fabrics
375 was observed. By increasing the fibre surface roughness and partial removal of the waxy
376 outer layer of the fibre with plasma, the cellulose became more accessible and more open to
377 contact with a cellulase enzyme. After an initial short period (about 10-15 min) of slow sugar
378 liberation, the results were described by straight lines. The slopes of the lines, applicable over

379 the latter period of time were 0.0024, 0.0024 and 0.0020 g/l·min for the plasma treated fabrics
380 for 30, 90 and 180 min, respectively, indicating that reducing sugars were liberated at
381 comparable rates by cellulase enzymes from the fabrics treated with plasma for different
382 times. It has to be noted that the slope of line for raw cotton was significantly lower (0.0008
383 g/l·min).
384



385

386 **Figure 6.** Reducing sugars released from raw, air-plasma (P) treated (for 30, 90 and 180 s)
387 raw and only bleached cotton fabrics by cellulase enzyme as a function of time of hydrolysis.
388

389 For comparison, Figure 6 also shows the reducing sugar data for cellulase hydrolysis
390 of the bleached cotton, which can be considered as pure (100 %) cellulose with perfect
391 accessibility. The slope of the line was 0.0045 g/l·min, indicating a much faster hydrolytic
392 degradation of cellulose into sugars. On the perfectly accessible cellulose surface of the

bleached cotton (obtained after removal of the cuticle and primary wall by alkaline scouring and bleaching), the cellulase enzyme digested gradually the secondary wall i.e. the main body of the fibre.

It has to be noted, that theoretically the reducing sugars liberated from the cotton fabrics by a cellulase enzyme can be derived not only from the fibres in weft yarns, as discussed above, but also from the warp yarns. Since the warp yarns are covered by a thick starch sizing layer, which can only be removed by a long hydrolysis (for 60 min and 120 min for plasma treated and raw fabric, respectively) with an amylase enzyme (Figure 4), it is likely that under the circumstances of the cellulase catalysed hydrolysis, the starch size still remained on the warp yarns. Thus, it is unlikely that the accessibility of the elementary fibres inside the warp yarns to the cellulase enzyme is high enough to enable the hydrolysis of cellulose.

Conclusions

In this paper, we focused on how the air-plasma treatment changed the surface of raw cotton fabric, and how these changes affected the enzyme reactions of cotton. Results proved that plasma slightly destroyed and oxidized the starch size on the surface of warp yarns and partially removed the thin and perfectly hydrophobic waxy coverage of the cotton fibres in the weft yarns, resulting in a more hydrophilic fabric with a significantly shorter wetting time and lower water contact angle. Plasma etching of the surface was accompanied by a creation of deep “pits” with a depth of 215 nm, which contributed to both the partial oxidation and removal of starch size from the warp yarns and the exposure of polymers such as cellulose and pectin located under the waxy outer layer of the fibres in weft yarns. Oxidation of the OH groups present in the cellulose and pectin molecules to aldehyde and carboxylic groups also occurred during the process. Furthermore, the rms roughness of the untreated elementary

418 cotton fibres (R_q 25 nm, which is similar to that previously reported in the literature[38]) in the
419 weft yarns of the fabric increased significantly to R_q 67 nm by a 180 s plasma treatment. The
420 fabrics' breaking load and elongation as well as dimensional stability, however, seemed to be
421 unaffected by the applied cold plasma treatment.

422 An amylase and cellulase digestion of the plasma treated cotton fabrics confirmed that
423 air-plasma significantly increased the accessibility of polymers (i.e. respective substrates) in
424 the fibres surface to the enzymes, resulting in an enhanced solubilization of both starch and
425 cellulose, respectively. Reducing sugars liberated from the starch-based size by amylase
426 treatment proved that about 10-20 % of starch size in the raw cotton was removed by the air-
427 plasma treatment. The increase in hydrophilicity and accessibility of the plasma etched
428 surface and the more efficient hydrolysis of residual starch resulted in an increased desizing
429 effect, which can lead to a significant shortening of the desizing process (from 60 to 10
430 minutes).

431 Raw cotton fibres in the weft yarns of the fabric had a low accessibility to cellulase,
432 which is due to the hindering effect of the waxy outer layer. Almost 30 minutes were needed
433 for the enzyme molecules to penetrate through the discontinuities of the wax to inner fibre
434 structures and attack cellulose. By plasma-aided degradation and removal of the waxy layer,
435 the accessibility of cellulose in the primary and secondary cell walls to cellulase enzyme
436 improved significantly and a dramatic increase in the rate of cellulose hydrolysis was
437 observed. Since the plasma treated substrates displayed significantly faster enzyme reactions,
438 the enzymatic treatment time can shortened sharply.

439

440 ## Acknowledgements

441 This work was supported by the Hungarian National Science Foundation (grant number
442 OTKA K82044).

References

1. Verschuren J, Kiekens P and Leys C. Textile-specific properties that influence plasma treatment, effect creation and effect characterization. *Text Res J* 2007; 77(10): 727–733.
2. Morent R, De Geyter N, Verschuren J, et al. Non-thermal plasma treatment of textiles. *Surf Coat Tech* 2008; 202: 3427-3449.
3. Zille A, Oliveria FR, Souto AP, Plasma treatment in textile industry. *Plasma Process Polym* 2015; 12(2): 98-131.
4. Szabo OE, Csiszar E and Toth A. Enhancing the surface properties of linen by non-thermal atmospheric air-plasma treatment. *Open Chem* 2015; 13(1): 570-576.
5. Trotman ER. *Textile scouring and bleaching*. London: Charles Griffin & Company Ltd., 1968, p.229.
6. Fu Kl and Lu DN. Reaction kinetics study of α-amylase in the hydrolysis of starch size on cotton fabrics. *J Text I* 2013; 105: 203-208.
7. Vigneswaran C, Anbumani N, Ananthasubramanian M, et al. Prediction of optimum process parameters to achieve eco-friendly desizing of organic cotton fabrics with indigenously produced alpha-amylase and their enzyme kinetics. *J Text I* 2012; 103(4): 422-433.
8. Li Y and Hardin IR. Treating cotton with cellulases and cectinases: Effect on cuticle and fiber properties. *Text Res J* 1998; 68(9): 671-679.
9. Presa P and Forte-Tavcer P. Low water and energy saving process for cotton pretreatment, *Text Res J* 2010; 80: 3-11.
10. Kalantzi S, Mamma D, Christakopoulos P, et al. Effect of pectate lyase bioscouring on physical, chemical and low-stress mechanical properties of cotton fabrics. *Bioresource Technol* 2008*;* 99: 8185-8192.

11. Csiszar E, Szakacs G and Koczka B. Biopreparation of cotton fabric with enzymes produced by solid-state fermentation. *Enzyme Microb Tech* 2007; 40: 1765-1771.

12. Grancaric A M, Pusic T and Tarbuk A. Enzymatic scouring for better textile properties of knitted fabrics. *J. Nat. Fibres* 2006; 2(3): 189-197.

13. Hardin IR and Li Y. Enzymatic Scouring of Cotton. *Textile Chem Color* 1997; 66(8): 71-76.

14. Forte-Tavcer P. Low-temperature bleaching of cotton induced by glucose oxidase enzymes and hydrogen peroxide activators. *Biocatalysis and Biotransformation* 2012; 30(1): 20-26.

15. Tomasino C, Cuomo JJ, Smith CB, et al. Plasma treatments of textiles. *J Ind Text* 1995; 25: 115-127.

16. Kan C-W, Lam C-F, Chan C-K, et al. Using atmospheric pressure plasma treatment for treating grey cotton fabric. *Carbohyd. Polym* 2014; 102: 167– 173.

17. Li X and Qiu Y. The effect of plasma pre-treatment on $NaHCO_3$ desizing of blended sizes on cotton fabrics. *Appl Surf Sci* 2012; 258: 4939– 4944.

18. Bhat NV, Netravali AN, Gore AV, et al. Surface modification of cotton fabrics using plasma technology. *Text Res J* 2011; 81: 1014-1026.

19. Pandiyaraj KN and Selvarajan V. Non-thermal plasma treatment for hydrophilicity improvement of grey cotton fabrics. *J Mater Process Tech* 2008; 199: 130–139.

20. Sun D and Stylios GK. Effect of low temperature plasma treatment on the scouring and dyeing of natural fabrics. *Text Res J* 2004; 74: 751-756.

21. Wang Q, Fan X-R, Cui L, et al. Plasma-aided cotton bioscouring: Dielectric barrier discharge versus low-pressure oxygen plasma. *Plasma Chem Plasma P* 2009; 29: 399–409.

491 22. Karaca B, Csiszar E and Bozdogan F. Effects of atmospheric plasma pre-treatments on
492 pectinase efficiency in bioscouring of linen fabrics. *Plasma Chem Plasma P* 2011; 31(4):
493 623-633.
494 23. Wong KK, Tao XM, Yuen CWM, et al. Effect of plasma and subsequent enzymatic
495 treatments on linen fabrics. *Color Technol* 2000; 116: 208-214.
496 24. Ibrahim NA, El-Hossamy M, Hashem MM, et al. Novel pretreatment process to
497 promote linen-containing fabrics properties. *Carbohyd. Polym* 2008; 74: 880-891.
498 25. Nithya E, Radhai R, Rajendran R, et al. Synergetic effect of DC air plasma and
499 cellulase enzyme treatment on the hydrophilicity of cotton fabric. *Carbohyd Polym* 2011;
500 83: 1652–1658.
501 26. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar.
502 *Anal Chem* 1959; 31: 426-428.
503 27. Song KH and Obendorf SK. Chemical and biological retting of kenaf fibers. *Text Res*
504 *J* 2006; 76: 751-756.
505 28. Csiszar E. Surface properties and residual chromophore content of differently
506 pretreated linen fabrics. *Text Res J* 2012; 82: 94-104.
507 29. Holme I and Au CK. The alkali desizing of woven cotton fabrics. *RJTA* 1999; 3: 16-
508 30.
509 30. Hari PK, Behera BK, Prakash J, et al. High pressure squeezing in sizing: Performance
510 of cotton yarn. *Text Res J* 1989; 59: 597-600.
511 31. Ryser U and Holloway PJ. Ultrastructure and chemistry of soluble and polymeric
512 lipids in cell walls from seed coats and fibres of *gossypium* species. *Planta* 1985; 163: 151-
513 163.
514 32. Wong KK, Tao XM, Yuen, CW, et al. Topographical study of low temperature plasma
515 treated flax fibers. *Text Res J* 2000; 70: 886-893.

516 33. Chung C, Lee M and Choe EK. Characterization of cotton fabric scouring by FT-IR
517 ATR spectroscopy. *Carbohyd Polym* 2004; 58: 417-420.
518 34. Laine J, Stenius P, Carlsson G, et al. Surface characterization of unbleached kraft
519 pulps by means of ESCA. *Cellulose* 1994; 1: 145-160.
520 35. Csiszar E and Fekete E. Microstructure and surface properties of fibrous and ground
521 cellulosic substrates. *Langmuir* 2014; 27: 8444-8450.
522 36. Buchert J, Carlsson G, Viikari L, et al. Surface characterization of unbleached kraft
523 pulps by enzymatic peeling by ESCA. *Holzforschung* 1996; 50: 69-74.
524 37. Calvimontes A, Mauersberger P, Nitschke M, et al. Effects of oxygen plasma on
525 cellulose surface. *Cellulose* 2011; 18: 803-809.
526 38. Wang L, Zhang Y, Gao P, et al. Changes in the structural properties and rate of
527 hydrolysis of cotton fibers during extended enzymatic hydrolysis. *Biotechnol Bioeng* 2006;
528 93(3): 443-456.
529