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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

25 Introduction

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

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

46 Plasma pretreatment can affect the removal of both sizing agent and waxy surface
47 layer. Cold-plasma in oxygen¹⁵, oxygen/helium^{16,17} and air¹⁸ atmosphere was highly effective
48 in removing the starch size from the surface of woven fabrics, resulting in a significant
49 increase in surface hydrophilicity. The maximum weight loss values measured in

50 oxygen/helium and air plasma were 2.92 %¹⁶ and 6 %¹⁸, respectively. The surface roughness
51 of the size layer characterized by the root mean square roughness (rms) increased significantly
52 (from 2.8 to 33.1 nm) with increasing the time of oxygen/helium plasma treatment (from 0 to
53 45 s, respectively).¹⁷ Furthermore, plasma pretreatment accelerated also the degradation of the
54 residual starch size in the subsequently applied desizing process with NaHCO₃.¹⁷ Plasma was
55 proved to be effective also in degradation of the waxy outer layer of cotton fibers.¹⁹ By the
56 effective removal of the waxy materials from the fibre surface with a short plasma
57 pretreatment, the processing time of the subsequently applied conventional chemical
58 processes was reduced significantly.²⁰

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

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

74

75 Experimental

76

77 *Fabrics and enzymes*

78 Raw cotton fabric, plain-weave, with a fabric weight per unit area of 153 g/m² and a
79 fabric count (warp/weft) of 28/24 yarn/cm was selected for the experiments. Warp yarns of
80 the fabric were sized by a standard starch-based sizing material. The fabric was cut to the
81 required dimension (10 cm × 20 cm, approximately 6 g) and these samples were then
82 conditioned at 65 % relative humidity and 20 °C for 24 hours prior to the plasma treatment
83 and any of the testing. Bleached cotton fabric (scoured with sodium hydroxide and bleached
84 with hydrogen peroxide) with a weight per unit area of 150 g/m², plain-weave, was selected
85 as a perfectly hydrophilic and accessible substrate with a cellulose content of nearly 100 %
86 and used exclusively in the cellulase enzyme hydrolysis without any pretreatment. Both
87 fabrics were supplied by Pannon-Flax Linen Weaving Co., Hungary.

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

96

97 *Plasma treatment*

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

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

103

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

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

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

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

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

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

131

132 *Analysis of the surface and bulk properties*

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

143 X-ray photoelectron spectroscopy (XPS) was done by a Kratos XSAM 800
144 spectrometer using Mg K $\alpha_{1,2}$ radiation and fixed analyser transmission mode (80 and 40 eV
145 pass energies for survey and detailed spectra, respectively). The diameter of the analysed spot
146 was about 2 mm. The spectra were referenced to the C 1s line (binding energy, BE=285.0 eV)
147 of the hydrocarbon type carbon. Data acquisition and processing were performed with the
148 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 $\pm 5\%$).

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 μm 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,

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

176

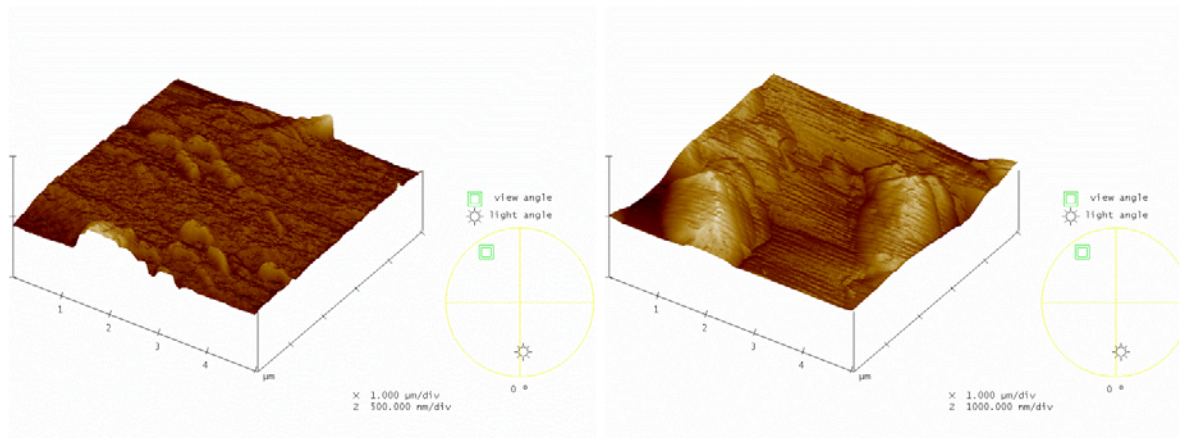
177 Results and discussion

178

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

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

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

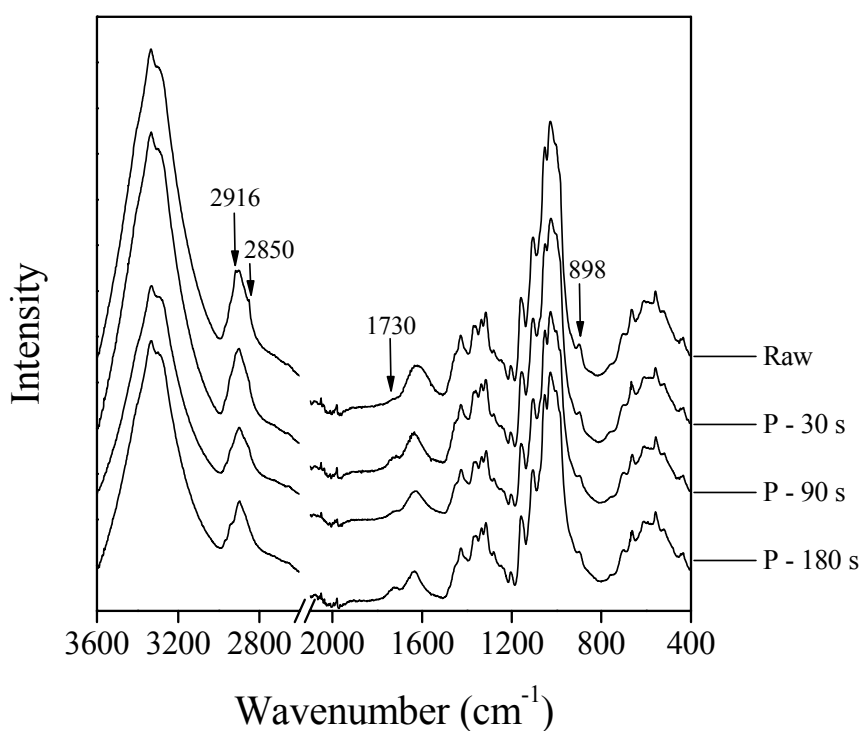


199
 200 **Figure 1.** AFM images of the elementary fibres of weft yarns derived from raw (left) and air-
 201 plasma treated cotton with exposure time of 180 s (right). Image scale: 5 µm in *x* and *y*
 202 directions and 500 nm in *z* direction.

203
 204 Changes in the waxy surface layer of the elementary fibres in the weft yarns were also
 205 proved by ATR FT-IR (Figure 2). Since the penetration depth of light is typically of the order
 206 of a few microns (ca. 0.5-3 µm), the results provided by FT-IR ATR were used successfully
 207 for characterization of the plasma-induced changes in constituents of the surface of
 208 elementary fibres in weft yarns. Extra peaks in the spectrum of the raw fabric at 2916 and
 209 2850 cm⁻¹, associated with the asymmetric and the symmetric stretching of methylene groups
 210 in long alkyl chains, indicate the presence of waxy materials in the fibre surface.³³ For plasma
 211 treated samples, however, the decrease in relative intensities of the peak at 2850 cm⁻¹ (Table
 212 1) and the absence of the peak at 2916 cm⁻¹ (Figure 2) was observed, indicating a partial
 213 removal of the waxy outer layer of the elementary fibres in weft yarns by plasma treatment.

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

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



225 **Figure 2.** ATR FT-IR spectra of the raw and air-plasma treated (P) cotton fabrics with
226 exposure time of 30 s, 90 s and 180 s.
227

228
229 As seen in Table 1, the O/C ratios for the plasma treated samples were significantly
230 higher (for 90 s: 0.54; for 180 s: 0.82) than for that of the raw cotton (0.25). Upon plasma
231 treatment, both components C2 (286.7 eV, carbon in C-OH and C-O-C) and C3 (288.3 eV,
232 carbon in C=O and O-C-O) increased (Figure 3 and Table 1). It is remarkable that the surface
233 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 cellulose³⁶. 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 reactions³⁷. 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

251

252 **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
 253 (raw) and air-plasma treated cotton fabrics as a function of time of plasma treatment.

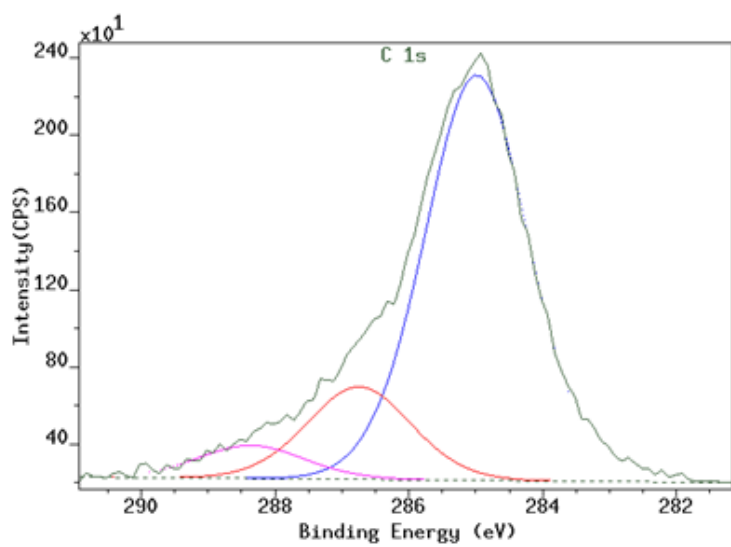
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255		Relative intensities at ^a				C1 %	C2 %	C3 %
256	Fabric samples	2850	1730	898	O/C	285.0 eV	286.7 eV	288.3 eV
257		(cm ⁻¹)				C–C, C–H	C–O	C=O, O–C–O
258	Raw	1.01	0.25	0.67	0.25	76	18	6
259	Plasma treated – 30 s	0.97	0.31	0.70	n.d. ^b	n.d.	n.d.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

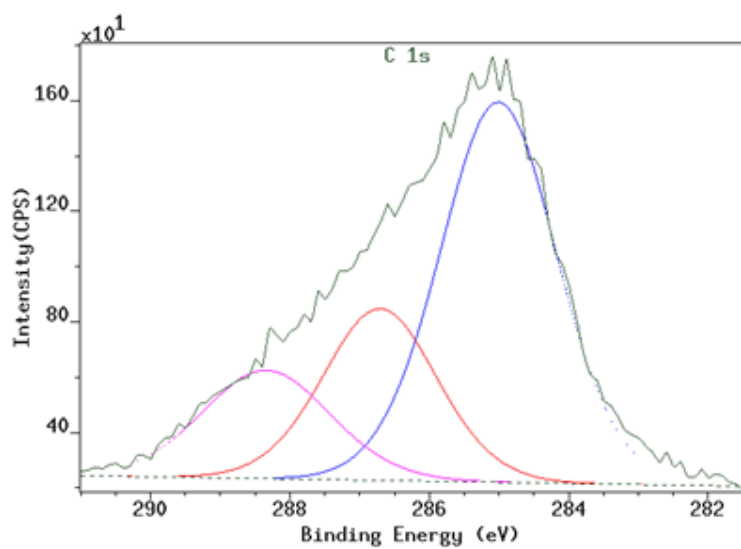
262 ^a compared to 609 cm⁻¹

263 ^bn.d. - not determined

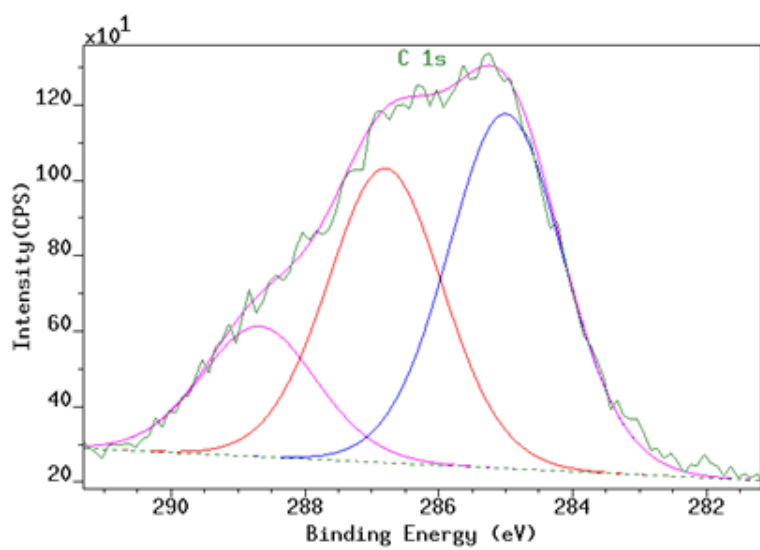
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266



267

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

270

271 Exposure of cellulose and pectin in the elementary fibres of weft yarns was confirmed
272 by ATR FT-IR (Figure 2). Significant changes were observed in the ester region at ~ 1730
273 cm^{-1} as well as at 898 cm^{-1} . The former is related to the pectin molecule, since approximately
274 70 % of carboxyl groups of galacturonic acid units in pectin are esterified with a methoxyl
275 group, and the latter is associated with the $\text{C}_1\text{-O-C}_4$ β -glycosidic bonds present in cellulose
276 molecule.^{8,25} Results in Table 1 proved that the relative intensities increased significantly by
277 plasma treatments (at 1730 cm^{-1} : 0.25 versus 0.31, 0.37 and 0.41; at 898 cm^{-1} : 0.67 versus
278 0.70, 0.72, 0.73; for raw versus plasma treated fabrics with exposure time of 30 s, 90 s and
279 180 s, respectively). This means that the partial degradation and removal of the waxy outer
280 layer of cotton, which occurred during the applied air-plasma treatment, exposed the pectin
281 and cellulose constituents and made them more detectable on the fibre surface by ATR FT-IR.

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

287

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

290

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

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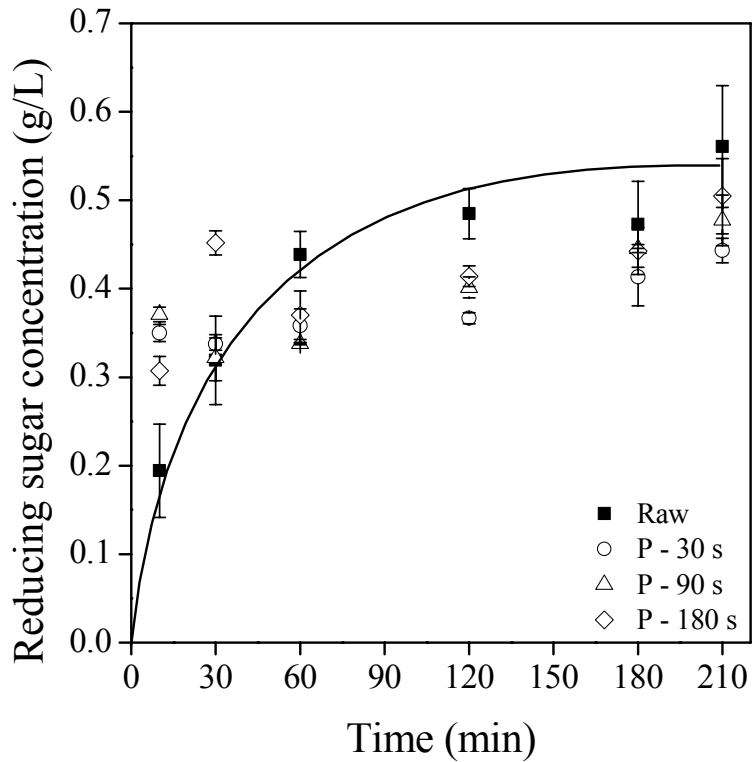
304 Since these changes in the surface, to an extent, could affect tensile properties of the
305 fabrics, an investigation was conducted to determine the breaking load and elongation of the
306 fabrics. Since any shrinkage and alteration in appearance of the fabrics did not occur during
307 the DCSBD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

312

313 *Enzymatic hydrolysis of starch size on the yarn surface*

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

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



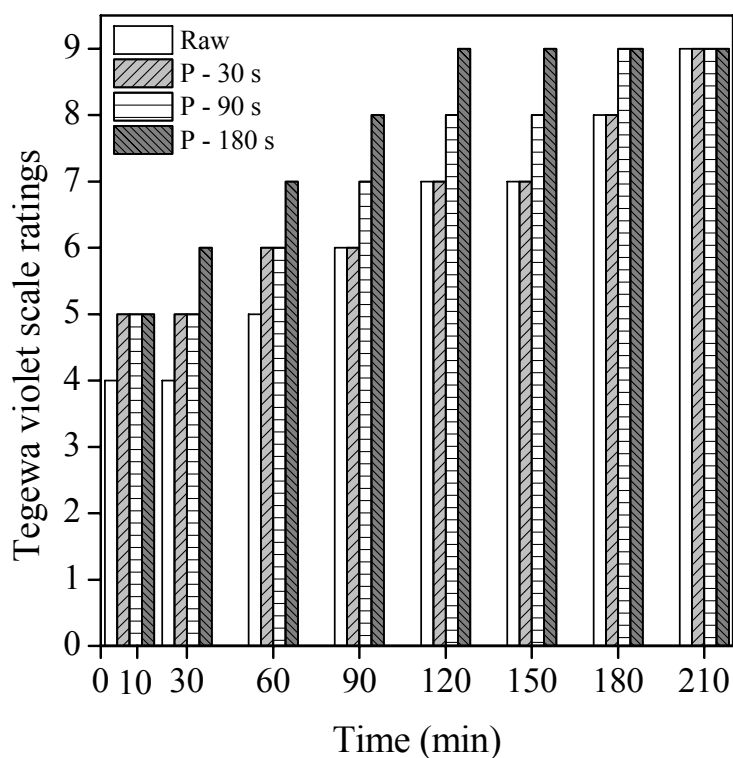
326

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).

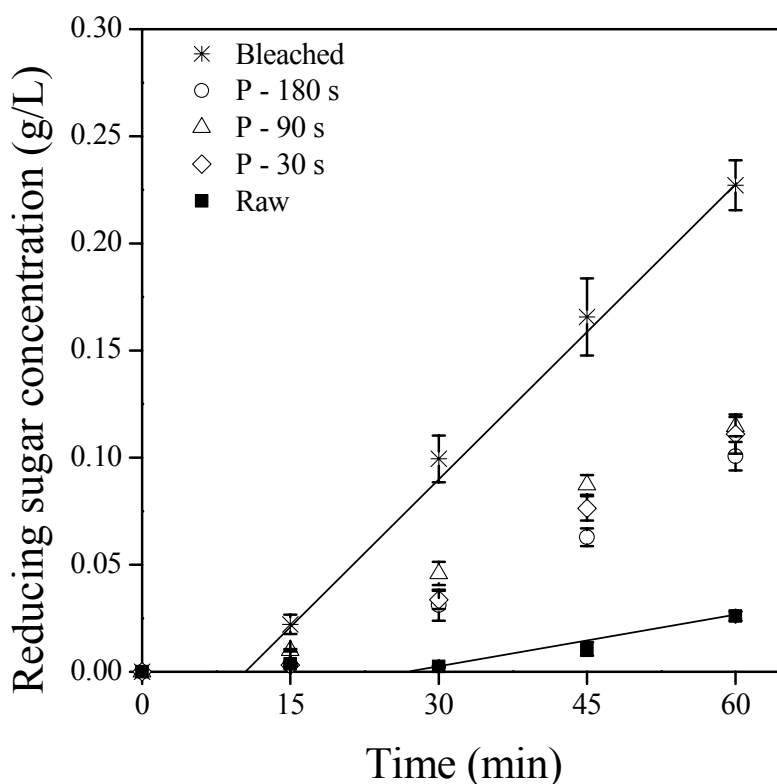
359 *Enzymatic hydrolysis of cellulose in the fibre surface*

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

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

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

405

406 Conclusions

407 In this paper, we focused on how the air-plasma treatment changed the surface of raw
408 cotton fabric, and how these changes affected the enzyme reactions of cotton. Results proved
409 that plasma slightly destroyed and oxidized the starch size on the surface of warp yarns and
410 partially removed the thin and perfectly hydrophobic waxy coverage of the cotton fibres in the
411 weft yarns, resulting in a more hydrophilic fabric with a significantly shorter wetting time and
412 lower water contact angle. Plasma etching of the surface was accompanied by a creation of
413 deep “pits” with a depth of 215 nm, which contributed to both the partial oxidation and
414 removal of starch size from the warp yarns and the exposure of polymers such as cellulose
415 and pectin located under the waxy outer layer of the fibres in weft yarns. Oxidation of the OH
416 groups present in the cellulose and pectin molecules to aldehyde and carboxylic groups also
417 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 literature³⁸) 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 be shortened sharply.

439

440 Acknowledgements

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

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