MOLECULAR WEIGHT OF CELLULOSE AFTER TREATMENT WITH A TOTAL CELLULASE

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Abstract

Gel Permeation Chromatography was used to compare peak, weight-average, and number average molecular weights of cotton fabric before and after treatment with a total cellulase preparation. Fabric was treated in either a Launderometer or a reciprocal water bath/shaker for times ranging from 10 to 180 minutes. Weight loss and breaking load measurements on control fabrics, buffer-only treated fabrics, and cellulase-treated fabrics were compared to molecular weight data. Despite considerable weight loss and breaking load reduction produced by treatment in the Launderometer with a total cellulase, GPC analysis did not show a reduction in molecular weight of the cellulose .

Introduction

The use of cellulase enzymes in modification of cotton fabrics has grown in recent years. Cellulases can be used to produce an aged appearance of denim (so-called 'enzymatic stonewashing'), to biopolish, and to soften cotton fabrics. Researchers have sought to optimize the effects of such hydrolysis, usually focusing on fabric physical properties such as tensile strength, weight loss, fuzzing, hand, copper number, moisture regain, and color change (in the case of dyed fabrics) (2, 5, 7, 9, 11).

There are three major components in cellulases: endo-glucanases, exo-glucanases (also known as cellobiohydrolases), and B-glucosidases (also known as cellobiases). Endo-glucanases cleave internal glucosidic bonds in the cellulose chain, producing shorter chains. Exo-glucanases produce cellobiose in a step-wise manner from the non-reducing end of the cellulose chains, and cellobiases hydrolyze cellobiose to glucose. A "total" cellulase preparation contains mixtures of these three types of enzymes (1, 6, 8).

Measurement of molecular weight (MW) and molecular weight distribution (MWD) of cellulose before and after treatment with cellulases offers the possibility of a direct assessment of effects of enzymatic hydrolysis on a fundamental property - length of the cellulose chains. Such information would help optimize the effects of cellulase hydrolysis, whereby the desired effects can be achieved while minimizing undesired effects such as excessive strength loss.

Gel Permeation Chromatography (GPC) has a long history of use in the determination of MW and MWD of synthetic polymers (3). GPC, also known as Size Exclusion Chromatography, is a separation technique whereby separation takes place based on size or hydrodynamic volume of a macromolecule relative to pore size of the stationary phase. Incorporation of both a differential refractometer and viscometer as detectors for GPC permits use of the universal calibration technique, whereby column calibration is valid for any polymer, regardless of its chemical nature; i.e., the narrow-distribution well-characterized standards used for column calibration need not be chemically identical to the material under study (4).

GPC requires dissolution of the material under study without degradation. For this reason it is preferable to avoid derivatization prior to dissolution, to prevent possible changes in the material. Relative MW of wood pulps has been determined on solutions of cellulose in dimethylacetamide with 0.5% w/v lithium chloride via GPC analysis using a differential refractometer (10, 12). This solvent, with incorporation of a differential viscometer to permit determination of absolute MW by universal calibration, was also used for characterization of cotton fiber MW/MWD (13-17). In the present study, we explore the use of GPC determination of MW and MWD to assess the effect of treatment of a cotton fabric with a total cellulase.

Experimental

Materials

Cellusoft L[1], a liquid total enzyme (Novo Nordisk) was used for the cellulase treatments at pH 4.8.

Fabric

The fabric was produced from Deltapine 50 cotton (Leland MS, 1989 growth season) as an 80x80 printcloth, 30's warp yarns, 36's fill yarns, desized, scoured, bleached, and acidified.

Enzyme treatments

Enzyme hydrolysis was done using either a New Brunswick Model R76 reciprocating water bath or an Atlas Electric Co. Launderometer. Treatments were at a pH of 4.8 maintained by a 0.05M acetate buffer, at a liquor:fabric ratio of 20:1 and an enzyme dose of 10% (w/w) OWF. Incubation time was 10, 60, or 180 min. Buffer-only controls were carried through each treatment.

Launderometer

Bias-cut 9x17" swatches, weighing approximately 10.5 g, were treated in 1200 ml SS Type 2 canisters (8.9x20.3cm) at 50ºC, with 50 SS ball bearings (~1/4" dia). The Launderometer was pre-heated to 50ºC at 5.5 degrees/min and held at that temperature for 2 min to equilibrate before

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loading the canisters. After treatment for the prescribed time, the enzyme was denatured by elevating the temperature to 75ºC at 5.5 degrees/min, holding at that temperature for 10 min. The canisters were cooled to 45ºC and the swatches were rinsed four times in 800 ml deionized water and air-dried.

Reciprocating water bath

Die-cut 3.5" circles of fabric, in approximately 23.5 g lots, were treated in 1 L wide mouth Erlenmeyer flasks at 50ºC, with a stroke length of 0.75" and a frequency of 120 strokes/min. Enzyme activity was quenched by addition of sufficient 30% (w/v) sodium carbonate to raise the bath pH to 10. Bath liquors were decanted and the fabric circles were rinsed three times in 1000 ml deionized water and air-dried.

Test Methods

Weight loss was measured on a dry weight basis. Fabric breaking strength was measured by ASTM Method D5035-90. Moisture regain was conducted in a textile conditioning room at 70ºF and 65% relative humidity, on samples previously dried at 60ºC under vacuum overnight.

Sample preparation for GPC

Control and enzyme-treated fabrics were Wiley-milled to 20 mesh. 50 mg of cotton was added to 5.0 ml dried DMAc in a 10 ml Reacti-vial heated to 150ºC in a Reacti-Therm heater/stirrer (Pierce Model 18970). The vial was uncapped for the first 15 minutes to allow any residual moisture to be driven off. After capping, the vial was maintained at 150ºC for 4 hr; temperature was reduced to 100ºC and after 20 min equilibration at 100ºC, 0.250g dried lithium chloride was added. The vial was mixed on a vortex mixer and returned to the heater/stirrer. The vial was alternately heated/stirred for 1 hr and shaken on a wrist-action shaker for 1hr, for 3 heat/shake cycles. The vial was then maintained at 100ºC with stirring overnight. The temperature was then reduced to 50ºC and the vial was maintained at that temperature with stirring overnight. Contents of the vial were quantitatively transferred to a 50 ml volumetric flask, and diluted to 50 ml (giving a final concentration of 1 mg cotton / ml in 0.5% LiCl in DMAc. The solution was filtered through a Millipore Millex SR-X 0.50µ syringe filter into a 4 ml autosampler vial. Triplicate aliquots of cotton were dissolved for each sample, with triplicate injections from each vial for a total of 9 determinations for each treatment.

GPC Analysis

Chromatography was carried out using a Waters 600E pump/controller Waters 717 autosampler, and a Waters column heater and temperature controller. Detection was via Viscotek Model 250 dual differential refractometer/viscometer, with a Viscotek Model 400 Data Manager interface, using Viscotek TriSEC GPC software Ver. 2.70. Mobile phase was 0.5% lithium chloride (Baker analyzed reagent grade, previously dried at 110ºC) in dimethylacetamide (DMAc, B&J chromatography/spectrometry grade, previously dried over molecular sieves). Stationary phase was three Polymer Labs PLGel Mixed B 10µ columns (each 7.5x300 mm) with a matched guard column. Flow rate was 1.0 ml/min; injection volume 150µl; run time 45 min. Column temperature was maintained at 80ºC; detector temperature was maintained at 30ºC.

Universal calibration

Polystyrene standards (Polymer Labs) with MW's ranging from 11,300 to 3,900,000 were dissolved in dimethylacetamide; eleven standards with duplicate injections were used. Viscotek's TriSEC Universal Calibration Module (ver 2.70) was used to produce the calibration curve, with an r^2 of 0.99991. The MW 3000 standard could not be resolved from the injection peak on the system used. Percent error in the calibration for predicted vs actual averaged 0.028% $\pm$ 2.3%.

Results

Peak MW (M_p, the molecular weight at the peak of the MWD), weight-average MW (M_w, the MW which an equal weight of sample molecules fall on either side of), number-average MW (M_n, the MW which an equal number of sample molecule falls on either side of), polydispersity (P_d, the ratio of M_w/M_n, a measure of the breadth of the distribution), weight loss, moisture regain, and breaking load are reported for treatments done in the Launderometer (Table 1) and for treatments using the reciprocal shaker (Table 2).

For fabrics treated in the Launderometer, buffer controls matched to each treatment time were analyzed. Weight loss of the buffer controls was negligible. Weight loss increased with time of hydrolysis, from 1.7% at 10 minutes to 19.7% at 180 minutes. Breaking load of buffer controls was reduced 5%, 0.6%, and 0.4% for treatment times of 10, 60, and 180 min, respectively. Cellulase treatment reduced breaking load of the fabric by 11%, 36%, and 77% for treatment times of 10, 60, and 180 min, respectively. Moisture regain was increased slightly by the buffer-only treatments, less so for the 10 min treatment than for treatments at 60 and 180 min. Cellulase treatments at 60 and 180 minutes increased moisture regain relative to the untreated controls, but less so than the buffer only.

M_p is the peak molecular weight of a polymer, the MW at the peak of the distribution curve. M_w, the weight-average molecular weight, can be visualized as the MW at which an equal weight of molecules falls on either side. Similarly, M_n, the number-average MW, is the MW at which an equal number of molecules falls on either side of the distribution. M_w is increased by a relative increase in proportion of larger molecules, and M_n is effected by a relative increase in proportion of smaller molecules. M_n of the buffer controls was increased slightly relative to the uncontrolled fabric. The 10 min enzyme treatment did not change M_n

significantly, and there were only slight changes in M_n for the 60 and 180 min treatments (Figure 1A). The pattern was similar for M_w (Figure 1B), with the largest increase for the 60 min enzyme treatment. M_p followed the same trend as M_w (Figure 1C). Polydispersity was not significantly changed by the treatments (Figure 1D).

Fabric treated in the reciprocal shaker had a negligible weight loss for the buffer only control. Weight loss after enzyme treatment was 1.3%, 2.2%, and 4.8% for treatment times of 10, 60, and 180 min, respectively. Breaking load was decreased only after the 180 min enzyme treatment, and then only by 4%. Buffer-only treatment increased moisture regain slightly, and enzyme treatments produced a smaller increase in moisture regain than did the buffer. Agitation was much lower in the reciprocal shaker than in the launderometer, and it is known that considerable mechanical agitation is required to achieve the desired effects of enzyme treatment (6). The lesser agitation in the shaker resulted in lower weight losses and less reduction of breaking load than did the more intense agitation in the Launderometer.

Treatment with the buffer only for 180 min increased M_n by a negligible amount, and there were negligible increases after the buffer treatments (Figure 2A). M_w was increased slightly by the buffer-only treatment, with slightly larger but insignificant increases after the enzyme treatments (Figure 2B). M_p was increased slightly by both the buffer and enzyme treatments (Figure 2C). Polydispersity was not changed significantly by the treatments (Figure 2D).

Discussion

Treatment of a cotton printcloth fabric with a total cellulase preparation in a reciprocal shaker resulted in smaller weight losses and reduction in breaking strength than did treatment in a Launderometer, demonstrating the effect of mechanical agitation in enzyme treatments. It has been suggested that increasing mechanical action during enzymatic hydrolysis, by decreasing enxyme adsorption, increases the number of free sites in the cellulose where the enzyme can attack, particularly for endo-glucanase-rich preparations (6).

Despite the considerable weight loss and breaking load reduction produced by treatment in the Launderometer with a total cellulase, GPC analysis did not show a reduction in molecular weights of the cellulose . This result supports the suggested mechanism that exo-glucanases (cellobiohydrolases) rapidly cleave cellobiose units from cellulose chains once the chains have been cleaved by endo-glucanases. In our system, MW's <3000 cannot be resolved from the injection peak. We hypothesize that the exo-gluconases degrade cellulose chains to components that are below the limits of measurement by GPC so rapidly that we were unable to detect the products. Although there was appreciable weight and strength loss, the cellulose chains remaining in the fabric after treatment were those not yet degraded by the total cellulase. There would be a reduction in the microfibrillar size, but such a reduction would not be detectable by GPC. This hypothesis will be tested in future studies using purified endo-glucanases, whereby one would expect to detect the larger subunits of the cellulose chains produced by the endo-glucanases in the absence of exo-glucanases.

Disclaimer

Use of a company and/or product name is for information only and does not imply approval or recommendation by the U.S. Department of Agriculture to the exclusion of others.

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Table 1. 80x80 Cotton Printcloth (DPL 50) 10% Cellusoft L owf; pH 4.8; 50°C; 20:1 Launderometer

	Control	Control	10 E[a]	10 B	60 E	60 B	180 E	180 B
Mn x 10^{-5}	3.9±1.5	4.6±1.1	**5.0±1.1**	6.0±2.1	**8.7±3.3**	6.2±2.4	**5.7±2.1**	3.6±1.4
Mw x 10^{-5}	11.8±2.8	11.8±2.6	**14.4±1.3**	12.3±4.7	**17.8±4.2**	13.6±3.2	**13.9±3.7**	10.7±3.4
Mp x 10^{-5}	8.0±0.9	8.6±1.3	**12.1±2.1**	10.3±4.3	**16.9±5.8**	11.8±3.1	**10.3±3.4**	8.5±2.4
Pd	3.4±1.8	2.7±0.5	**3.0±0.8**	2.1±0.4	**2.2±0.6**	2.3±0.6	**2.6±0.7**	3.3±1.3
MR,%	6.00±0.01	____	**6.12±0.02**	6.11±0.02	**6.11±0.01**	6.13±0.01	**6.10±0.002**	6.13±0.02
WtL,%	----	----	**1.73**	0.06	**6.57**	0.14	**19.69**	0.27
BL,lb	53.2	----	**47.2**	50.4	**34.1**	52.9	**12.2**	53.0

[a] E = Enzyme/buffer treatment B = buffer only treatment

Table 2. 80x80 Cotton Printcloth (DPL 50); 10% Cellusoft L owf; pH 4.8; 50°C 20:1 Reciprocal Shaker

	Control	Control	180 B[a]	10 E	60 E	180 E
Mn x 10^{-5}	3.9±1.5	4.6±1.1	6.1±2.4	**5.6±2.8**	**5.2±2.8**	**5.3±1.4**
Mw x 10^{-5}	11.8±2.8	11.8±2.6	14.3±3.0	**17.5±4.3**	**17.9±5.1**	**16.1±4.0**
Mp x 10^{-5}	8.0±0.9	8.6±1.3	12.6±3.5	**15.0±6.0**	**12.9±1.8**	**11.6±3.7**
Pd	3.4±1.8	2.7±0.5	2.8±1.5	**3.9±2.3**	**3.8±1.7**	**3.1±0.7**
MR, %	6.12±0.04	____	6.23±0.02	**6.18±0.03**	**6.16±0.002**	**6.16±0.02**
WtL, %	----	----	0.30	**1.27**	**2.19**	**4.78**
BL, lbs	49.8	----	55.0	**56.1**	**53.4**	**47.9**

[a] E = Enzyme/buffer treatment B = buffer only treatment

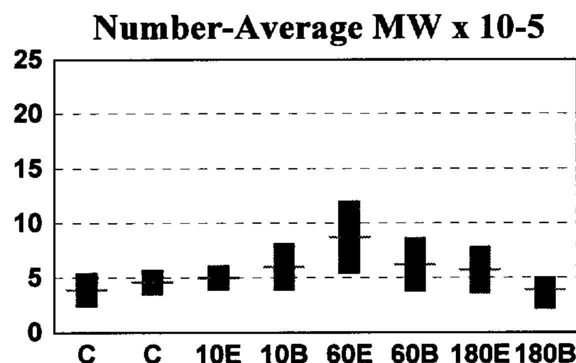

Figure 1A Launderometer

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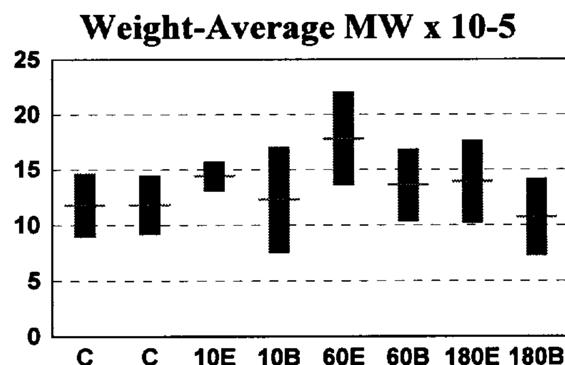

Figure 1B Launderometer

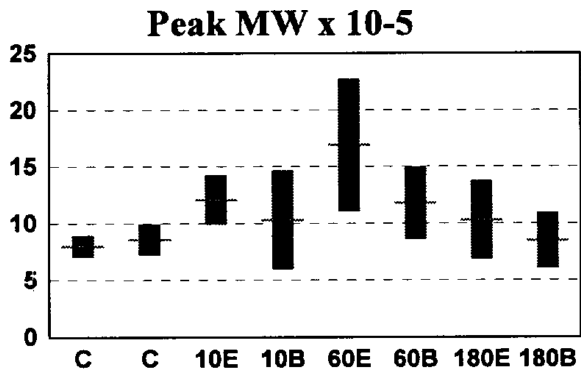

Figure 1C Launderometer

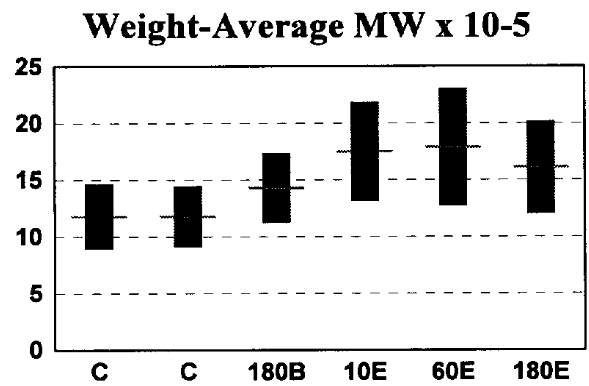

Figure 2B Reciprocal Shaker

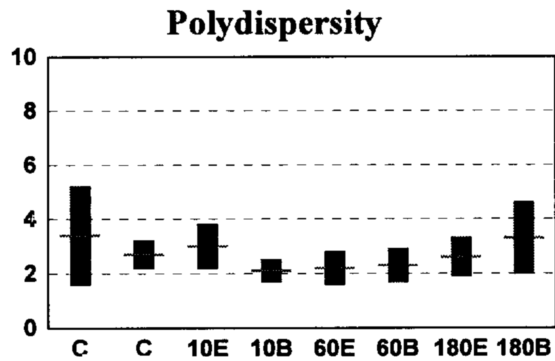

Figure 1D Launderometer

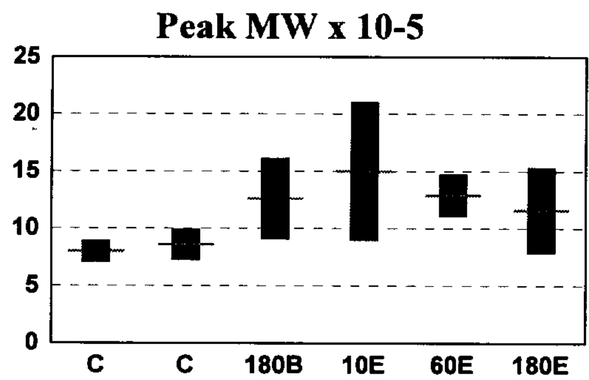

Figure 2C Reciprocal Shaker

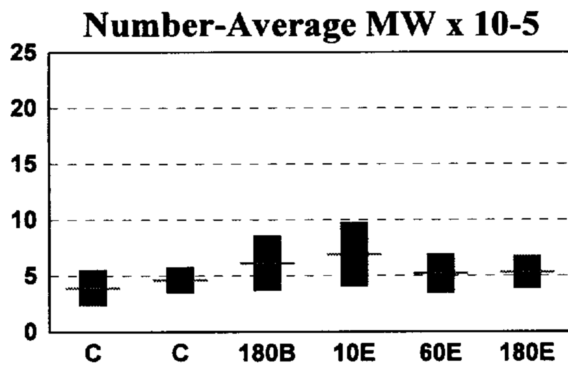

Figure 2A Reciprocal Shaker

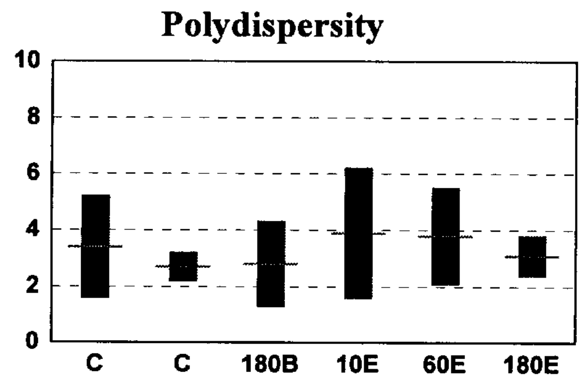

Figure 2D Reciprocal Shaker