# EFFECTS OF SEQUENTIAL TREATMENT OF CELLULASES AND PROTEASES ON DEVELOPING COTTON FIBERS

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## Abstract

A series of oligomeric glycans can be extracted from the cell walls of developing cotton fibers with weak acid. Glycans that produce similar profiles on high pH anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) are also found in a protein complex extracted from developing fibers (Murray, *et. al*., 2001). Treatment of the extracted oligomers with cellulase from *Trichoderma reesei* results in almost complete degradation of many of the smaller oligomers in the series and significant degradation of the larger oligomers with a concominant increase in the smaller oligomers and glucose. The oligomers can be released from the protein complex either by weak acid or by proteases. Subsequently intact fibers were treated with enzymes to determine if it was possible to remove the oligomers by specific enzymatic means. Such a result would suggest a role for the oligomers as components of the fiber cell wall. Immature fibers (25 days post anthesis) were incubated for 24-hrs at 37°C with trypsin, chymotrypsin, proteinase K or pepsin, followed by a second 24-hr incubation at 37°C with cellulase (*T. reesei*) or ß-glucosidase. Alternatively, samples were incubated in the reverse order with cellulase or ß-glucosidase treatment followed by the protease. Certain early eluting oligomers were released from fibers by both proteases and cellulase. The residual material was then treated with 0.1N HCl at 100°C and the extracts analyzed by HPAEC-PAD.

Fibers treated with protease followed by cellulase and and subsequently treated with weak acid resulted in many of the oligomeric glycans and a residue of apparently intact fibers. Treatment of the same fibers in reverse order with cellulase followed by protease resulted in breakdown of the fibers and only the first eluting oligomer. Many of the oligomers of the series were extracted from fibers incubated with protease first followed by cellulase. Chymotrypsin was the most effective protease at releasing oligomers and degrading fibers. When mature fibers from opened bolls were subjected to the same cellulase followed by protease procedure, there was little effect, therefore the procedure was repeated. At the end of the second cycle, the fibers disintegrated and only a fine particulate precipitate remained. This precipitate was washed, and digested sequentially in 0.1N HCl, 2N trifluoroacetic acid or 6N HCl at 100°C. Degradation only occurred in 6N HCl, indicative of crystalline cellulose. The resulting monosaccharide composition was at least 99% glucose. The cellulase from *T. reesei* was compared to cellulases from *T. viride* (two sources), *T. longibrachiatum, Aspergillus niger* and *Humicola insolens.* Only the cellulases from *T. reesei* and *T. longibrachiatum* were effective in making the fibers susceptible to protease degradation, yet the cellulase from *T. viride* completely degrades the isolated oligomers.

## Reference

Murray, A.K., R. L. Nichols, and G. F. Sassenrath-Cole, 2001, Phytochemistry, 57(6):975-986.

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