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DIFFERENTIATION OF COTTON CULTIVARS BY OPTIMIZED EXTRACTION AND CHROMATOGRAPHIC ANALYSIS OF GLYCAN OLIGOMERS

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Abstract

A series of oligomeric glycans can be extracted from the cell walls of developing cotton fibers with weak acid. The same and similar glycans have also been extracted from developing and mature cotton fiber, cotton fabric, wood from different species, various types of paper products and marine algae. While many of the oligomers isolated from the various cellulose sources display the same peaks when analyzed by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD), the specific numbers of oligomers and their relative quantities appear unique for each source of cellulosic material. Preparation of cotton fiber samples has been improved by homogenization of tissues using micromechanical means (Pro 200 Homogenizer with Micro-Gen tip); and the variability among the samples reduced by optimizing extraction volumes to compensate for the lower solubility of oligomeric products with higher degrees of polymerization (DPs). Using the refined sample preparation and extraction procedures, differences in the oligomer profile between samples of mature fibers of the cotton cultivars 'DP-50' and 'Ultima' have been demonstrated. In addition, the unique oligomeric profile of Ultima was preserved during processing from raw cotton to finished fabric.

Introduction

A series of glucose containing oligomers, can be extracted from developing cotton fibers, mature cotton fibers and cotton fibers in new and washed fabric, and appear to be ubiquitous in all plant tissues (Murray, et. al., 2001, Murray, 2003). The quantitative distribution and presence of specific oligomers appears to be unique to each plant and tissue as well as to the developmental stage. We hypothesize that these glycans constitute part of the cell wall biosynthetic process (Murray et al. 2001).

In order to obtain maximum utility from the information obtained from the analysis of these oligomers, their extraction must be optimized and variability between samples must be minimized. The amount of each oligomer extracted from cotton fibers depends in part on the ratio of the extract volume to the mass of fiber in the sample. As expected, the oligomers of lower molecular weight are somewhat more soluble than are the larger oligomers. However, variability in results, possibly due to tissue preparation from sample to sample, has been a concern. . We hypothesize that thorough homogenization of fibers is essential to achieve quantitatively reproducible extractions of the higher molecular weight oligomers. We report here the use of a new homogenization technique. As a result the improved tissue preparation, the oligomeric analysis has demonstrated differences between fibers of two cultivars and the conservation of geometric similarity of the oligomeric profiles of one cultivar through processing from fiber to finished fabric.

Materials and Methods

The cotton, (*Gossypium hirsutum*) cultivars DP-50 and Ultima , were grown in Mississippi , and in the San Joaquin Valley of California, respectively. A bale of Ultima was shipped to Cotton Incorporated, the fiber processed using manufacturing-scale equipment, and a knit fabric made. In the previous method of sample preparation, mature cotton fibers or a fabric sample were diced as finely as possible with a razor blade. A 5-mg sample of fibers was placed in a 2.0 ml screw cap plastic tub and 0.5 ml of water was added. In the refined method, the sample was homogenized with a PRO 200 Homogenizer with a Multi-Gen tip (PRO Scientific, Inc.), 7-mm in diameter, until a uniform homogenate was obtained. Using either sample preparation method, the tube was shaken, then placed in a Branson 85 W sonicator filled with ice water for 15 minutes.. The tubes were centrifuged at 15,000xg for 5 minutes. The supernatant was removed with a Pasteur pipette, 1.0 ml of 0.1 N HCl was added and the tube was mixed on a vortex mixer and placed in a boiling water bath for 30 min to extract the glucose containing oligomers. HPAEC-PAD was performed using a CarboPac PA-1 column. The eluent was 150 mM sodium hydroxide, isocratic from 0 to 5 min then a linear sodium acetate gradient from 5 to 40 min going from 0 to 500 mM in 150 mM NaOH at a flow rate of 1 ml/min. The waveform had a potential of +0.1V from 0 to 0.40 sec, -2.0V from 0.41 to 0.42 sec, +0.6V from 0.43 to 0.44 sec and –0.1V from 0.44 to 0.50 sec with integration from 0.20 to 0.40 sec.

Results

Two experiments were done on mature fibers that were finely diced with a razor blade. In a preliminary experiment, the amount of each early-eluting oligomer increased with additional extraction volumes from 0.5 to 1.5ml of 0.1 N HCl. Therefore a subsequent experiment was performed using 0.5, 1.0, 1.5, 3.0 and 6.0 ml of 0.5ml of 0.1N HCl on 10-14mg fiber. The results of this second experiment were expressed as the total amount of oligomer extracted/mg of sample. Together the two experiments indicated that the maximum extraction was achieved at 1.5ml. (Data not shown)

A PRO 200 homogenizer with 7mm tips was used in subsequent experiments. The homogenizer does not work well unless the fibers are first finely chopped with a razor blade. In addition to the chopping, the ratio of fiber to liquid is important. Good results were achieved with up to 5mg of fiber in 0.5ml of extractant in a 2-ml tube, but it is important to visually examine the tube to be certain that good homogenization has been obtained. Therefore, further refinement of the method may be necessary to understand its best utilization with different types of cellulosic materials. Oligomer profiles of DP-50 and Ultima fibers were very reproducible. The difference between DP-50 and Ultima fibers is shown in Figure 1. Once good reproducibility had been obtained, Ultima fibers at seven stages between raw cotton and finished fabric were extracted. The oligomer profiles of Ultima at the seven stages are shown in Figure 2. The characteristic oligomer profile of Ultima appears to be preserved throughout each processing stage and in the finished fabric.

Discussion

In this laboratory, investigation of the soluble sugars extracted from freeze-dried developing and mature cotton fibers determined that mechanical disruption of fibers did not make a significant difference in the amount of sugars extracted (Murray, 1998). This lack of difference is presumably due to the high solubility of the sugars and the disruption of membranes by the freezing and drying processes. Subsequent studies on the glycan oligomers extracted from developing cotton fibers raised questions concerning the efficiency of tissue disruption caused solely by means of freeze drying. The oligomers are less soluble than the simple sugars, and weak HCl is required to release them. Moreover, we presume that they are involved in the process of cell wall assembly (Murray, 2000, Murray, et. al., 2001, Murray, 2003). In order to achieve greater quantitative precision in the data on the oligomers extracted from cotton fibers, issues of oligomer solubility and tissue heterogeneity were addressed. Based on the results of the reported experiments, the ratio of extraction volume to tissue mass was increased to optimize solubility of the larger oligomers. The structure of cotton fibers that makes them useful for yarn and fabric also renders them very difficult to uniformly homogenize. To date finely chopping the fibers with a razor blade followed by the use of the Pro 200 homogenizer has been found to be the best method to prepare cotton fibers for extraction.

Improved homogenization of cotton and increased solubility of the oligomers in the tissue extracts has permitted the use of chromatographic analysis to illustrate differences in oligomeric profiles between two cotton cultivars. One goal of this research is to correlate oligomeric profiles of fibers with fiber properties measured by other means. The further purpose is to gain insight into the biochemical structure of the fibers, and the relationship of fiber structure to fiber properties. Such information should be useful to cotton breeders for fiber improvement. The demonstration that the unique oligomeric profile of the fibers are preserved through processing to finished fabric is encouraging, and should extend the utility of this type of analysis to applications in textile processing.

Acknowledgement

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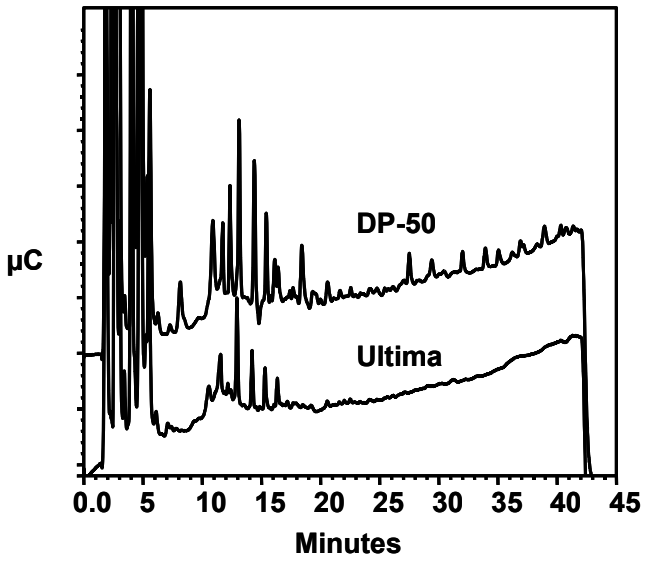

Figure 1. Oligomer profiles of DP-50 and Ultima.

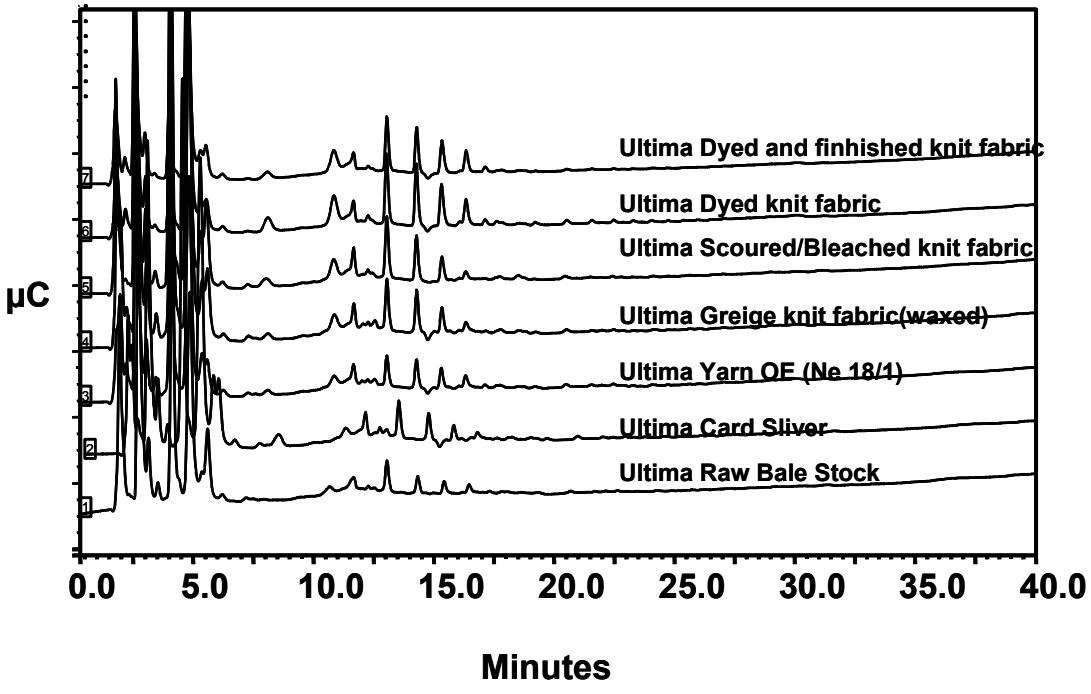

Figure 2. Oligomer profile of Ultima at different stages between raw cotton and finished fabric.