SAMPLE COLLECTION METHODS FOR CARBOHYDRATE ANALYSIS IN DEVELOPING COTTON FIBERS

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Abstract

In the investigation of carbohydrate metabolism of developing cotton fibers it is essential to stop metabolic events as rapidly as possible on harvesting the bolls. To address the question of possible post-harvest physiological events bolls were subjected to four different freezing protocols in the field. Bolls were sealed in vacuum bags and then kept at ambient temperature for one hour or on ice for one hour before being placed on dry ice. Alternatively, bolls were placed directly on dry ice or placed in a dry ice/isopropanol bath for 1-2hr before being placed in a dry ice chest for storage. Samples were then freeze dried and extracted with water for carbohydrate analysis. The ambient temperature samples showed signs of post-harvest metabolic activity. As expected, the faster the samples were frozen the better the results. The optimal method was that of immersing the samples in a dry ice/isopropanol bath, immediate placement on dry ice was the next best and both of these methods were better than for samples placed on ice for 1 hour before freezing.

Introduction

Carbohydrate analysis of developing cotton fibers has been employed in this laboratory (Murray, 1996, 1998, Murray and Brown 1996, 1997, Murray *et. al.* 1997, 1999). The carbohydrate analyses of developing cotton fibers are characterized by a high degree of variability for the content of soluble mono- and oligosaccharides when expressed on a per mg fiber basis. Although this variability may be inherent in field samples, the possibility that the manner inn which the samples are collected may be a contributing factor. The specific question of the rate of freezing of the collected bolls was investigated. Four different methods of freezing field samples were employed.

Methods

Cotton plants, Maxxa, were grown at the University of California, West Side Research and Extension Center. Collected bolls were sealed in Food Saver™ plastic bags using a Food Saver™ vacuum sealing device. The portable 110v power source for the Food Saver™ consisted of a deep cycle marine/RV 12V battery connected to a 600W inverter. The sealed bags were handled by four different methods. In the "ambient" method allowed the bags to remain at ambient temperature for 1-1.5 hr before being placed in a dry ice chest with 2 in slabs of dry ice. In the "ice" method, the bags were placed in an ice/water bath for 1-1.5 hr before being placed in a dry ice chest with 2in slabs of dry ice. In the "dry ice" method the bags were placed directly in a dry ice chest with 2in slabs of dry ice. In the "dry ice/isopropanol" method, the bags were immersed in a dry ice/isopropanol bath for 1-2 hr before being placed in a dry ice chest with 2in slabs of dry ice. Frozen bolls were transported to the laboratory and freeze dried. Cotton fibers were subjected to aqueous extraction and analysis of the soluble carbohydrates by high pH anion chromatography with pulsed amperometric detection (HPAEC-PAD) (Murray, 1998). Additional extraction of the oligomers (~mers) was achieved under conditions of dilute acid and elevated temperature prior to HPAEC-PAD (Murray, 2000).

Results

The soluble oligosaccharides extracted from fibers from bolls cooled by the "ambient" and "ice" methods are shown in Figure 1. Due to the inherent variability in the fibers, three analyses are show for "ambient" and two are shown for "ice". Approximate retention times are the following: melibiose, 6.1min, sucrose, 8.5min, raffinose, 13.25min, stachyose, 13.6min and verbascose, 14.1min. The internal standard, maltotriose, has a retention time of 16.4min. The peak at 11.1min in the "ambient" method is an unidentified peak, which appears during *in vitro* incubation of cotton fibers. The soluble oligosaccharides extracted from fibers from bolls cooled by the "dry ice" and "dry ice/isopropanol" methods are shown in Figure 2. In all cases except the "dry ice/isopropanol" method, the resolution of raffinose, stachyose and verbascose is not as sharp as in the "dry ice/isopropanol" method. In addition, the "dry ice/isopropanol" method produces a sharp leading edge on the raffinose peak, which is not seen in the other treatments.

In some samples analyzed in this laboratory the rounded leading edge or unresolved leading edge of the raffinose peak has been seen. This rounded peak has been observed with a number of peaks eluting between 15-25min. This extraction was scaled up and the leading edge collected and re-chromatographed where it produced a sharp peak as shown in Figure 3. The collected peak was then extracted with chloroform, taken to dryness, taken up in water and re-chromatographed along with the aqueous phase as shown in Figure 4. An expanded scale chromatogram of the chloroform extract is shown along with the original chromatogram in Figure 5. These results indicate that a series

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of later eluting peaks can present as an unresolved diffuse peak on the leading edge of the raffinose peak under these conditions. The series of later eluting peaks are also observed with *in vitro* incubation of fibers.

Discussion

As expected, the "ambient" method of cooling harvested bolls is the least desirable. However, even bolls cooled on "ice" and "dry ice" result in chromatograms which are not as sharply resolved as are the chromatograms of extracts of bolls from the "dry ice/isopropanol" method. It is also apparent that products from post-harvest physiological events can result in a poorly resolved leading edge on the raffinose peak.

Summary

The optimal method for freezing harvested cotton bolls involves rapid vacuum sealing in a plastic bag, which is then immersed in a dry ice/isopropanol bath.

References

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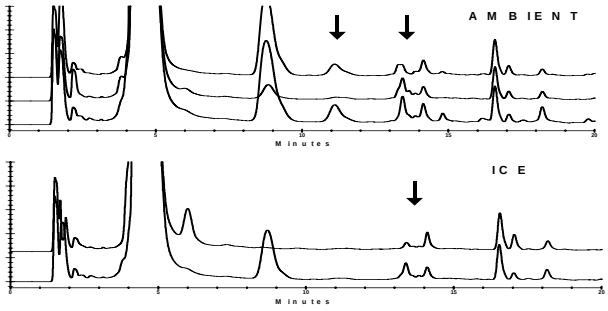

Figure 1. Soluble oligosaccharides extracted from cotton fibers from bolls kept at ambient temperature or on ice for one hour before freezing.

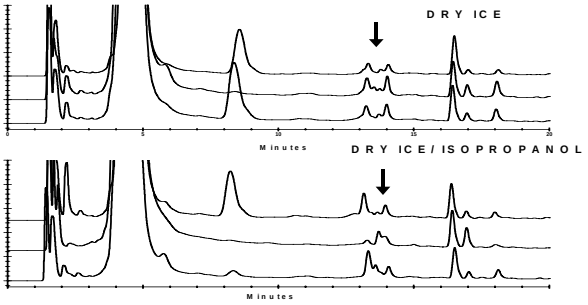

Figure 2. Soluble oligosaccharides extracted from cotton fibers from bolls placed directly on dry ice or in a dry ice/isopropanol bath.

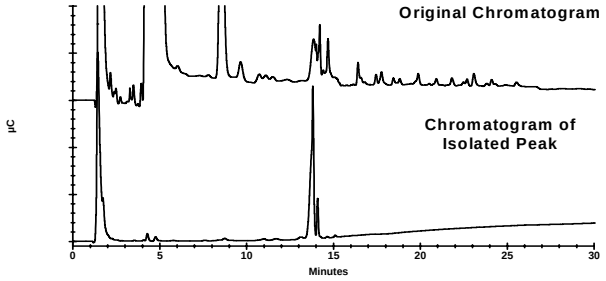

Figure 3. Scaled-up extraction and isolated peak.

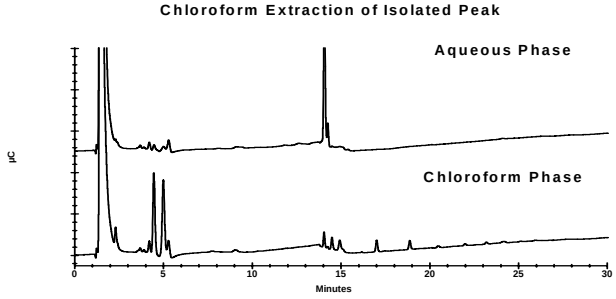

Figure 4. Chromatograms of aqueous phase and chloroform phase of isolated peak.

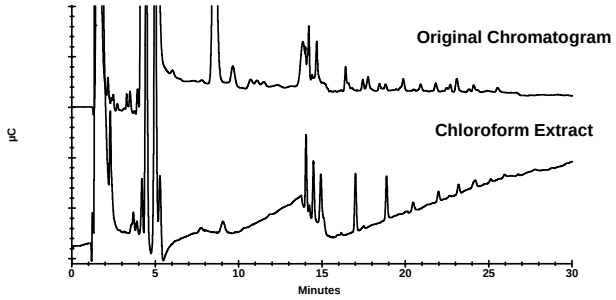

Figure 5. Original chromatogram and expanded scale of chromatogram of chloroform extract of isolated peak.