# ION CHROMATOGRAPHY SEPARATION OF COTTON SURFACE MELEZITOSE AND RAFFINOSE: ENTOMOLOGICAL VS. PLANT SUGARS

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## Abstract

According to previous studies, certain levels of the carbohydrates melezitose and trehalulose deposited on the surface of cotton are indicative of either whitefly or aphid contamination, which may cause problems during cotton processing. Obtaining reliable IC values for those surface sugars is paramount in diagnosing a contamination source. Since the plant sugars raffinose and sucrose are isomers of melezitose and trehalulose, respectively, it can be difficult to fully separate them from the entomological sugars via IC, especially when the analysis time is shortened. An improved IC method has been developed which separates melezitose and raffinose from one another; in total nine sugars were separated using the new method. By testing the water extracts of five raw cotton samples via our IC method, we have shown decreases in melezitose amounts present, when compared to the previous method. The difference in melezitose amounts were from separating out the raffinose and another unidentified component from the melezitose peak during IC integration.

## Introduction

Surface carbohydrates may give information about the developmental stage of cotton, carbohydrate metabolism differences amongst different cotton varieties, and the propensity of processing issues caused by stickiness.(Ellsworth et al., 1999; Rathert, 1983) The extent of a stickiness problem relies on many factors and is likely a complex interaction between physical and chemical properties: the type of sugar contamination, the type of insect responsible for contamination, the occurrence of immature fibers and the presence of waxes, salts and amino acids.(Barton, Bargeron, Gamble, McAlister, & Hequet, 2005)

Metabolically produced insect sugars melezitose and trehalulose, occur dominantly (in total sugar content) in either aphid (*Aphis gossypii*) and whitefly (*Behmesia argentifolii*) honeydew, respectively.(Hequet & Abidi, 2006) Some of the cotton's physiological sugars, such as inositol, glucose, fructose, sucrose, maltose and trehalose can be found in insect honeydew in differing amounts as well, but they have not been previously found to cause acute stickiness related issues; however, build-up of these sugars on processing equipment may occur.(Hequet & Abidi, 2006) Previously research has been done to determine that when certain ratios of entomological sugars are present on the cotton (usually ~35-40% of either trehalulose or melezitose, with specific mixtures of other carbohydrates), the pest contaminating the crop can be identified.(Hendrix, Wei, & Leggett, 1992; Hequet & Abidi, 2006; Peralta, 2015)

High performance anion exchange chromatography (IC) is the technique most effective for both identification and quantification of individual carbohydrates.(Abidi & Hequet, 2005; Hequet & Abidi, 2006; Ouchemoukh, Schweitzer, Bey, & Djoudad-Kadji, 2010) By comparing more reliable melezitose concentration values to enhanced trehalulose concentration values, a dependable ratio of the pest sugars can be obtained to reveal the most likely cause of sugar contamination. We have developed an IC method that can be used concurrently with physical testing methods as a measure in better identifying contaminated cotton; thus potentially allowing for a treatment effort, if at all possible, before full-scale problems arise at the processing mill.

## Experimental

Five raw cotton samples exhibiting a range of stickiness, as determined by physical Minicard testing, were obtained from the USDA-ARS-SRRC in New Orleans, LA. The five samples were named LOW A, LOW B, LOW C, MODERATE, and HEAVY; which denotes the level of stickiness determined by the previous Minicard physical

testing. All samples were measured in triplicate. For carbohydrate analysis via IC, 1 gram of cotton was used. The raw cotton sample was placed in a centrifuge tube, where 20 mL of ultrapure deionized water was added. The cotton/water sample was capped and vortexed. The water extract samples was then filtered through a 0.2 µm syringe filter to remove any cotton fibers and particulate, and then it was placed into the auto-sampler for analysis on the ion chromatography instrument.

Ion Chromatography was performed on a Dionex DX-5000 instrument using pulsed amperometric detection and two Dionex CarboPac PA-1 (4 × 250 mm) columns connected in series. The analytics software used was Dionex Chromeleon 7.2 CDS, which was purchased from Thermo Scientific. There were two instrumental methods used for comparison:

1.) The "GRADIENT IC" Method: elution was carried out using a flow rate of 0.75 mL/min. A sigmoidal gradient was employed whereby the following mobile phase solutions were used: A.) 200 mM NaOH, B) 200 mM NaOH/500 mM Sodium Acetate and C) 50 mM NaOH. Eluent A was decreased from [100% A/0% B] to [50% A/50% B] during the span of 0 to 6 minutes. The gradient continued from [50% A/50% B] to [100% C] over the span of 6 to 12 minutes. The column and compartment temperatures were set at room temperature.

2.) The "IMPROVED IC" Method: elution was carried out using a flow rate of 0.80 mL/min. An isocratic eluent delivery was employed for 28.5 minutes at a set temperature.

A stock standard matrix of eight of the sugars (inositol, trehalose, glucose, fructose, sucrose, melezitose, raffinose and maltose) was created in water. Calibration curves were created. The trehalulose standard stock was prepared and run on the IC separately, since the trehalulose syrup was only 90%.

## Results and Discussion

### Comparison of Ion Chromatography Instrumental Methods for Analysis of Water Extracted Sugars from Cotton Fibers

Distinguishing between melezitose and raffinose (found in cotton seed meat) is difficult because they differ only in sequence and linkage.(Enjalbert et al., 2013; Zhu, Bendiak, Clowers, & Hill, 2009) Trehalulose and the physiological sugar sucrose, which are also structural isomers, present the same type of separation concerns.(Hequet & Abidi, 2006) A previously published method analyzed for melezitose and trehalulose, however it did not appear that baseline resolution was fully obtained; more importantly, raffinose was not present in the analysis (Figure 1)(CIRAD, 2014).

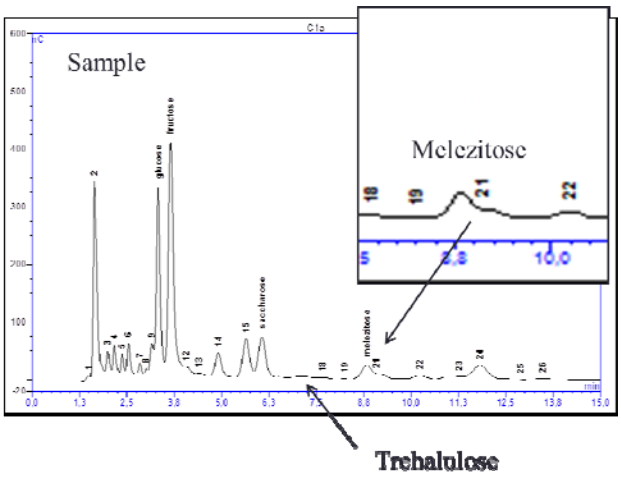


Figure 1: IC chromatogram of a previously published isocratic IC carbohydrate separation.

We also attempted the exact gradient method used by another research group, to the best of our knowledge, and due to the extensive bunching of peaks when using our instrumentation, we decided to employ a lower ionic strength solution of 50 mM NaOH toward the end of the sample run to allow for some relief of the peak overlapping. We noticed that we were able to attain retention times more in line with some previous methods; we termed this modified contemporary gradient method: GRADIENT IC. Again, this method did not allow for baseline resolution of the trehalulose/sucrose and melezitose/raffinose isomers; as raffinose was not accounted for as far as we know.

Five raw cotton samples were subject to water extractions and analyzed on the IC; whereby a sigmoidal gradient involving 200 mM sodium hydroxide, a solution of 200 mM sodium hydroxide/500 mM sodium acetate and a 50 mM solution of sodium hydroxide were employed over the course of 14.5 minutes.(Hequet & Abidi, 2006) It is interesting to note that the chromatogram obtained via a modified gradient method for sample HEAVY in Figure 2 shows overlap of trehalulose/sucrose and melezitose/raffinose as well as a significant baseline shift.

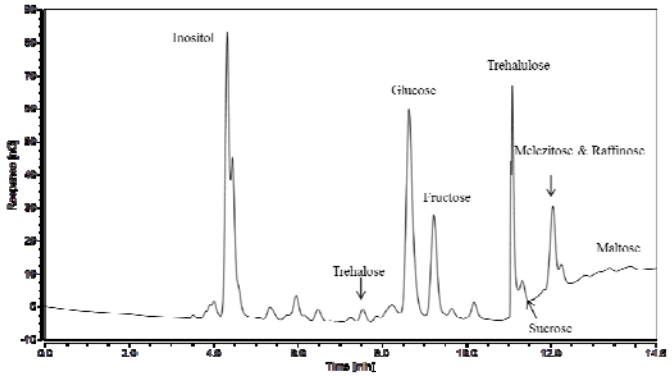


Figure 2: IC chromatogram for sample HEAVY obtained via the GRADIENT IC instrumental method.

**Modified Isocratic IC Instrumental Method**

Five raw cotton samples were subject to water extraction and tested on the IC using an updated, improved separation method whereby an isocratic delivery of NaOH was employed over the course of 28.5 minutes at an elevated temperature. Figure 3 shows a sample chromatogram of the nine sugar standards with trehalulose, sucrose, melezitose and raffinose fully separated with baseline resolution.

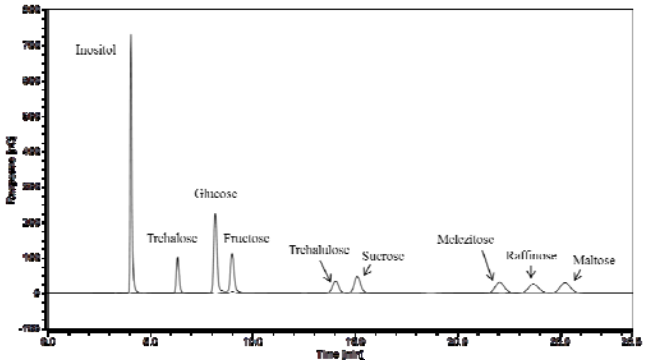


Figure 3: IC chromatogram of nine sugars present in 0.1 mg/mL concentrations obtained via the IMPROVED IC instrumental method.

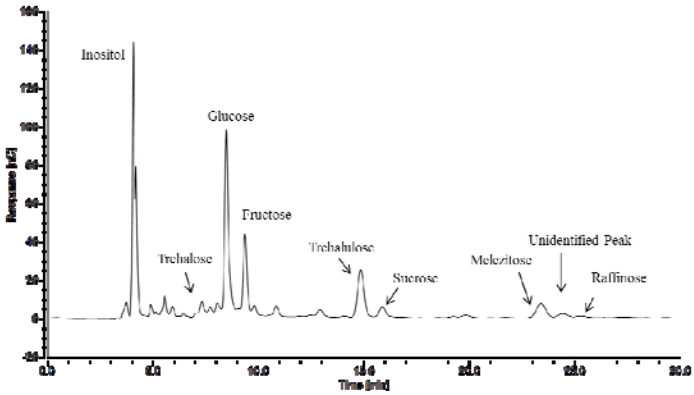


Figure 4: IC chromatogram for sample HEAVY obtained via the GRADIENT IC instrumental method.

Figure 4 shows the result of using our improved isocratic method on the same HEAVY sample, which was analyzed via the gradient method in Figure 2. Upon integration, it was found that the melezitose decreased, with the highest amount of melezitose actually remaining in a sample that was only ~22% of the original value given via the GRADIENT IC method. Raffinose increased from 0 mg using the gradient method to 0.049 mg for the HEAVY sample. Because of the raffinose increase, it is evident that the raffinose peak was adding to the melezitose retention peak to give false, increased milligram values. The unidentified peak at ~24.5 minutes was exacerbating the false high mg values for the melezitose in the samples as well. The trehalulose value increased using the IMPROVED method, while sucrose decreased. This could indicate that some of the trehalulose was erroneously integrated as sucrose when there was no baseline resolution between the retention peaks.

Using the IC software was difficult when using the GRADIENT IC method as baseline shifts (both positive and negative in value) were an issue. The differences in the baseline stability can be clearly seen when comparing Figures 2 and 4. The extremely large total average decrease in the melezitose (~91 %) and increase in trehalose (~132%, which is created by whiteflies) amongst all five cottons must be acknowledged as indicative of a qualitative shift in insect identification.

## Summary of Results

Our improved isocratic instrumental technique allow for more enhanced chromatographic differentiation between the melezitose and raffinose present on the cotton's surface. By changing to an IMPROVED IC isocratic method, we have shown baseline resolution of nine sugars of interest for cotton samples, as well as the separation and quantitation of raffinose. We also obtained baseline resolution between the isomers sucrose and trehalulose. We have successfully demonstrated that utilizing a GRADIENT method may cut down the analysis time on the IC, but retention peak overlaps can cause discrepancies in correctly identifying entomological and plant sugars. For our particular samples tested, melezitose amounts which may previously have pointed to a misdiagnosis of an aphid contamination were corrected, via our IC separation and integration, and shown to have a higher level of possible whitefly produced trehalulose.

## Acknowledgements

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## Disclaimer

The use of a company or product name is solely for the purpose of providing specific information and does not imply approval or recommendation by the United States Department of Agriculture to the exclusion of others.

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