MEASUREMENT OF SUGAR ON RAW COTTON BY HPLC, INDIVIDUAL CARBOHYDRATE CONCENTRATIONS AND THEIR RELATIONSHIP TO STICKINESS POTENTIAL

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Abstract

Sugars occur on raw cottons through two sources. The first, normal plant sugars are part of the growing process. As many as 10 known carbohydrates have been identified to be present in normal plant sugar extracts from cotton. The most predominant of these carbohydrates are the monosaccharides glucose and fructose. The second source, honeydew (insect sugars) occurs in the form of highly sticky droplets of more complex concentrated carbohydrates on the surface of cotton. Extracts from honeydew cottons contain normal plant sugars as well as the sucrose isomer trehalulose and a trisaccharide melezitose. Work using anion High Performance Liquid Chromatography (HPLC) has made it possible to separate, characterize, and quantify these sugars. Identifying specific carbohydrates and determining their overall contributions to cotton stickiness potential is an important step in developing intervention methods to alleviate cotton stickiness experienced in textile processing. The relationship between individual sugar concentrations; specifically honeydew sugars, and the USDA reducing sugar measurement is explored.

Introduction

All harvested cottons have sugars on their surfaces that are a product of the natural growing process. These 'plant sugars' generally vary from 0.1 to 1.00 percent on the fiber based on the lint weight. Major factors affecting sugar concentrations on lint are specific growing area, variety, weathering history, and microbial activity. If levels of these sugars on the cotton approach 0.5 percent or greater, long term accumulation of sticky materials on textile processing equipment, particularly card crush rolls, can cause frequent stoppage to clean parts, hence increased production cost. Common plant sugars, when broken down to individual carbohydrates contain primarily the monosacchrides glucose and fructose, the disaccharides sucrose and trehalose, and small amounts of other monosacchardies and oligosaccharides (2,15,16).

A second source of sugars on raw cottons comes from insect contamination commonly known as 'honeydew'. These sugars are more complex in composition than plant sugars. Concentrations on cotton vary depending upon the species of insect, the population, the species of plant upon which the insect is feeding, and weathering history (1,5,8). Honeydew is randomly deposited on the surface of cotton lint in highly concentrated droplets which are easily detected as sticky by the difficulty experienced in all phases of processing from the gin to the textile mill. Deposits on rolls, blades, and other equipment make some cottons virtually impossible to process. Shut down and clean-up is costly. In addition to the normal plant sugars, honeydew contaminated cottons contain the tri-saccharide melezitose, and the sucrose isomer trehalulose. Honeydew from the highly destructive silverleaf whitefly or "B strain" of the sweet potato whitefly, *Bemisia tabaci*, typically contains large amounts of trehalulose (2,3,4,5,8,9,). Aphid honeydew contaminated cottons contain little or no trehalulose.

Cotton stickiness as related to honeydew contamination has been a serious problem in certain growing areas in the western United States both in ginning and textile processing, particularly in the last 10 years or so (12,13). A recent survey indicates that insect infestation is perhaps moving to new growing areas (4). Methods for detection and characterization of honeydew on cotton is essential to the development of effective intervention techniques to reduce stickiness. One major test developed that is quickly becoming the accepted method for determining degrees or levels of stickiness in randomly deposited sticky spots on insect contaminated cotton lint is the GRAF/IRCT thermodetector (TD). By counting the number of sticky specks deposited from a web of cotton spread between two sheets of aluminum foil at a fixed temperature and time under applied pressure, cotton stickiness is determined. Using TD data and other information, current insect resistance studies are under way in affected areas to reduce the influences of honeydew.

Recent work with anion high performance liquid chromatography (HPLC) has been successful in separating and characterizing carbohydrates on the surface of cotton (2,3,4,6,7,8,9,10). With selective programming, proper eluant choices, and authentic calibrations of sugars previously identified as found on cotton, a routine method for quantitating and characterizing these carbohydrates has been developed (2,4). This report examines an extensive collection of HPLC analyzed cottons that are both insect and non-insect contaminated, their specific carbohydrate contents, relationships to stickiness as measured by thermodetector, and the reducing sugar content as determined by the routine reducing sugar test.

Description of Test Methods

Reducing sugar analysis on raw cotton lint samples were run using the United States Department of Agriculture (USDA) potassium ferricyanide test (11,14). These aqueous extractions were titrated manually using an endpoint indicator system calibrated with known

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concentrations of glucose standards within defined limits before any unknowns were tested. Standard deviations on replicate samples were ± 0.01 percent for reducing sugar concentrations up to 0.60 percent, ± 0.02 percent for 0.61 to 0.75 percent reducing sugar, ± 0.05 percent for 0.76 to 1.10 percent, and ± 0.10 percent above 1.10 percent reducing sugar.

The GRAF/IRCT thermodetector test for stickiness potential was determined on all of the cottons. Counting the number of sticky specks on two sheets of aluminum foil deposited by a 2.5 gram web of cotton determines a stickiness rating. Stickiness rating are divided into 4 levels. Nonsticky cottons are those depositing 4 or less sticky spots. Slightly sticky cottons deposit from 5 to 14 specks, moderately sticky lint between 15 and 24 specks, and heavily sticky cottons above 24 specks. Replicate (5 determinations per cotton) determinations at different levels of stickiness yield standard deviations of ± 1.0 speck up to 10 specks, ± 1.2 specks from 11 to 20 specks, ± 2.0 specks from 21 to 25 specks, and ± 5 specks above 25 specks in the extremely sticky range. Each lint sample was preconditioned at a relative humidity between 55 and 65 percent at least 24 hours before testing. The values reported in this data set are from single determinations on each cotton.

High Performance anion liquid chromatography was performed using a Dionex Series DX-300 system a described earlier (2). Analysis time for unknown lint extracts, including calculations of specific carbohydrate concentrations generally takes about 45 minutes per sample. Using an auto-sampler and running periodic calibration standards to correct for possible variations in column and detector sensitivity, it is possible to analyze up to 20 cottons daily. Values for individual carbohydrate levels for each cotton sample in this report are based in a single chromatogram from randomly selected 2 gram samples.

Uncontaminated Cottons

Normal cottons with only plant sugars on the surface have been found to contain at least 10 identifiable sugars. Generally, if the overall sugar content is low or as high as 1.0 percent, the same sugars are present, but in larger quantities. The four most prevalent sugars in cotton plant sugars are glucose, fructose, and the disaccharides sucrose and trehalose. These have been determined to account for at least 65 percent of total known carbohydrate present in non-insect contaminated raw cottons. Smaller amounts of the carbohydrates myo-inositol, arabitol, mannitol, arabinose, mannose, and turanose have also been identified. Turanose was present in high sugar cotton, but not in low sugar cotton. Glucose levels range from 1.4 to 2 times greater than fructose levels. Sucrose levels normally are slightly lower than fructose concentrations, however; studies of tests made on cottons from the 1994 and 1995 crops indicate an area of growth dependence. Table 1 is a summary of at least 15 random samplings each from the 1994 and 1995 crops in five United States growing areas. Average reducing sugar concentrations ranged from 0.19 percent in the eastern harvest area to a high of 0.35 percent in the High Plains of Texas. Glucose levels were found to increase as total reducing sugar content increased. Ratios of glucose to fructose concentrations were more area dependent. Average sucrose levels appear to be slightly higher in eastern and central cottons.

Thermodetector stickiness tests on these cottons were negative in the sense that they were rated as non-sticky. TD tests on certain uncontaminated cottons with high plant sugar contents sometimes deposit very small evenly distributed fibers on the test aluminum foil, but are easily recognized as very different in size and randomness as seen with insect contaminated cottons.

Whitefly Contaminated Cottons

A typical HPLC chromatogram of an extract from a whitefly contaminated cotton is shown in figure 1. The labeled peaks (1 through 6) are trehalose, glucose, fructose, trehalulose, sucrose, and melezitose, respectively. The sugars trehalulose (4) and melezitose (6) are sugars found only on insect contaminated cotton extracts. Combined, the above sugars normally account for at least 85 percent of known carbohydrates extracted from whitefly contaminated cottons. A survey of over 100 raw cottons from experimental western plots from 1995 and 1996 cotton crops contaminated with varying levels of whitefly honeydew were collected and analyzed. The "honeydew" sugars trehalulose and melezitose ranged from 10 to 37 percent and 4 to 22 percent of total sugars, respectively. Glucose concentrations varied from 10 to 26 percent and fructose levels from 19 to 40 percent of total known sugar extracts. The disaccharides trehalose and sucrose varied from a low of 2 percent to a high of 12 percent of total sugar each.

The most predominant and certainly the greatest contributing sugar to stickiness in whitefly contaminated cottons is trehalulose. Thermodetector stickiness numbers for over 100 whitefly contaminated cottons in this study varied from slightly sticky (a low of 5 sticky specks) to extremely sticky (a high of 44 specks). Calculated reducing and HPLC sugar concentrations ranged from a low of 0.38 for slightly sticky cottons to highs approaching 2.0 percent for very sticky cottons.

A comparison of titrated reducing sugar content and calculated HPLC sugars determined from calibrations with authentic carbohydrates would be a good test of HPLC data reliability. Figure 2 is a plot of 100 whitefly cotton extracts and their respective reducing and HPLC sugar contents. The coefficient of simple correlation for this relationship is 0.87.

In whitefly honeydew sugar extracts, any time the trehalulose concentration exceeds 1 percent; there is some

melezitose present. Since both sugars were found as exclusive to whitefly honeydew and in all of these cottons, their combined concentrations were compared to the reducing sugar content. When the reducing sugar content was less than 0.40 percent, the average trehalulose/melezitose concentrations were about 20 percent of total sugars or less. As reducing sugars increased, the trehalulolse/melezitose concentrations also increased. At reducing sugar levels in excess of 1.0 percent, the total trehalulose/melezitose concentrations were 35 percent or better. The coefficient of simple correlation for the data was 0.80.

The relationship between the honeydew sugars trehalulose and melezitose in whitefly cotton extracts for over 100 cottons is shown in figure 3. At a 10 percent concentration of trehalulose, the average concentration of melezitose is about 5.7 percent. At a higher level of trehalulose of 35 percent, melezitose averaged about 20 percent. The linear ratio up to 40 percent trehalulose is 1.75/1. A coefficient of simple correlation for this data was determined to be 0.94.

Unlike normal non-insect contaminated cottons, where the glucose concentration exceeds that of the fructose, as the trehalulose concentrations in whitefly contaminated cottons increase the glucose/fructose ratio changes. At a trehalulose level of approximately 10 percent of extractable sugars, glucose/fructose concentrations are basically the same. As the trehalulose concentration increases, the ratio changes in favor of fructose. For example, the fructose/glucose ratio is about 2/1 at 20 percent trehalulose and 2.5/1 at 30 percent. Approximately 50 percent of the cottons in this study had trehalulose levels between 30 and 40 percent of total sugars, hence their fructose/glucose ratios were at least 2.5/1.

Reducing sugar contents when compared to corresponding TD measurements for these cottons is shown in figure 4. The positive relationship with a coefficient of simple correlation of 0.92, suggests that generally when whitefly honeydew is present, reducing sugars between 0.30 and 0.70 percent would be expected to be slightly sticky (5 to 14 sticky specks), moderately sticky (15 to 24 sticky specks) between 0.70 and 1.1 percent reducing sugar, and extremely sticky above 1.1 percent reducing sugar.

Since a very high correlation was found between reducing sugar levels and total trehelulose and melezitose concentrations, it follows that whitefly honeydew sugar concentrations should also be positively related to TD stickiness. Figure 5 shows this data. When the total honeydew sugar levels approach 50 percent, considerable scatter in TD measurements were seen. Replicate TD measurements may have aided in smoothing out these results at higher stickiness levels. The coefficient of simple correlation for this data is 0.81. Numerically, slightly sticky cottons (5 to 14 specks) coincide with trehalulose/melezitose concentrations between 12 and 30 percent, moderately sticky (15 to 24 specks) between 30 and 46 percent, and extremely sticky cottons would have total trehalulose/melezitose levels approaching and above 50 percent of total sugars. In terms of trehalulose concentration, extremely sticky whitefly contaminated cottons would have concentrations approaching and above 30 percent trehalulose.

Aphid Contaminated Cotton

Aphid infested cottons do not follow the same honeydew contamination pattern as whitefly cottons. The limited inventory that we have analyzed in our laboratories have been found to contain little or no trehalulose and less than 5 percent melezitose. Reducing sugar concentrations generally range from 0.20 to 0.40 percent, however; these cottons are usually found to be extremely sticky on the TD. If there is one distinguishing characteristic of aphid honeydew, the melezitose level is higher than the trehalulose. Aphid honeydew contains a number of high molecular weight components that have not been identified. More work on other samples of aphid honeydew is required.

Summary

Large numbers of cottons from different growing areas for two recent harvest seasons have been analyzed for reducing sugar content, and individual carbohydrate concentrations by anion HPLC. Extracted cottons containing only plant sugars have the same sugars at all reducing sugar levels. Quantities of these sugars are dependent upon growing area, weathering history, and microbial activity. Whitefly contaminated raw cottons have the honeydew sugars trehalulose and melezitose in definite proportional amounts. There is a very positive relationship between reducing sugar content and whitefly honeydew sugar levels. As trehelulose concentrations approach 30 percent reducing sugar measurements are normally above 1.0 percent based on lint weight.

TD stickiness potential is directly related to whitefly honeydew content. Slightly sticky cottons usually occur when total honeydew sugar contents are between 12 and 30 percent, moderately sticky cottons between honeydew sugar contents between 30 and 46 percent, and extremely sticky cottons above 46 percent honeydew.

Reducing sugar contents are closely related to TD stickiness measurements for whitefly contaminated cottons. Most of the cottons in this study having reducing sugar contents between 0.30 and 0.70 were rated as slightly sticky by TD. Whitefly cotton reducing sugar contents in excess of 1.10 percent are generally rated as extremely sticky by TD.

A limited amount of work has been done on aphid contaminated cottons partially because of a limited inventory of samples. It has, however; been established that aphid honeydew is not like whitefly honeydew.

There appears to be little or no trehalulose present and small levels of melezitose.

References

Bates, R.B., D.N. Byrne, V.K. Vinayak, W.B. Miller, S.R. Taylor. 1990 N.M.R. characterization of trehahulose from excrement of the sweet potato whitefly, Bemisia tabaci Carbohydrate Research 201:342-345.

Brushwood, D.E. and H.H. Perkins, Jr. 1994. Characterization of sugar from honeydew contaminated and normal cottons. Proc. Beltwide Cotton Conferences, National Cotton Council, Memphis, TN. pp 1408-1411.

Brushwood, D.E. and H.H. Perkins, Jr. 1995. Variations in cotton insect honeydew composition and the related effects on test methods and processing quality. Proc. Beltwide Cotton Conferences, National Cotton Council, Memphis, TN. pp 1178-1181.

Brushwood, D.E. and H.H. Perkins, Jr. 1996. Cotton plant sugars and insect honeydew characterizated by high performance liquid chromatography. Proc. Beltwide Cotton Conferences, National Cotton Council, Memphis, TN. pp 1310-1313.

Byrne, D.N. and W.B. Miller. 1990. Carbohydrates and amino acid composition of phloem sap and honeydew produced by Bemisia tabaci. Journal of Insect Physiology 36:433-439.

Chatterton, N.J., P.A. Harrrison, W.R. Thorney, and J.H. Bennett. 1992. Separation and quantification of fructon (inulin) oligomers by anion exchange chromatography. Proc. 2nd International Conference on Inulin.

Hardy, M.R., R.R. Townsend, and Y.C. Lee. 1988. Monosaccharide analysis of glycoconjugates by anion exchange chromatography with pulsed amperometric detection. Analytical Biochemistry 170, pp 54-62.

Hendrix. D.L., Y.A. Wei, and L.E. Leggett. 1992. Homopteran honeydew sugar composition is determined by both the insect and the plant species. Comp. Biochem. Physiol. 101B:23-27.

Hendrix, D.L., B. Blackledge, and H.H. Perkins, Jr. 1993. Development of methods for detection and elimination of insect honeydews on cotton fiber. Proc. Beltwide Cotton Conferences, National Cotton Council, Memphis, TN, pp 1600-1603.

Hendrix, D.L. and Y.A. Wei. 1993. Bemsiose: an unusual tri-saccharide in Bemisia honeydew. Carabohydrate Research.

Perkins, H.H., Jr. 1971. Some observations on sticky cottons. Textile Industries. 135:4:49-64.

Perkins, H.H., Jr. 1991. Cotton stickiness - a major problem in textile processing. Proc. Beltwide Cotton Conferences, National Cotton Council, Memphis, TN, pp 523-524.

Perkins, H.H., Jr. 1993. Controlling cotton stickiness - summary of progress. Proc. 21st International Cotton Conf., Bremen, Germany, pp 219-224.

Perkins, H.H., Jr. 1993. A survey of sugar and sticky cotton test methods. Proc. Beltwide Cotton Conferences. National Cotton Council, Memphis, TN, pp 1136-1141.

Roberts, C.W. H.S. Koeing, R.G. Merrill, P.S.R. Chueng, and H.H. Perkins, Jr. 1976. Implications of monosaccharides in sticky cotton processing. Textile Research Journal, 46:374-380.

Roberts, C.W., P.S.R. Cheung, and H.H. Perkins, Jr. 1978. Implications of monosaccharides in sticky cotton processing. Part II, Effects of growing conditions on fiber contaminants. Textile Res. J. 48:91-96.

Table 1. Average carbohydrate concentrations from 1994/95 non-insect contaminated cottons.

Growing Area	Reducing Sugar %	Glucose %	Fructose %	Sucrose %	Trehalose %
Eastern	0.19	25.4	14.2	14.9	11.2
Central	0.28	35.1	22.5	11.3	11.1
Texas	0.30	25.4	14.2	14.9	11.2
Texas/ H.Plains	0.35	34.3	23.9	7.8	12.4
Western	0.29	29.1	17.9	9.6	9.0