HONEYDEW SUGAR ESTIMATES DIFFER AMONG REDUCING-SUGAR TEST METHODS

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Abstract

Physical methods such as the Sticky Cotton Thermodetector (SCT)(Shirley-CIRAD) predict stickiness in cotton lint contaminated by insect honeydew better than do available chemical test methods. However, U.S. textile mills still use reducing sugar tests to screen bales for stickiness more frequently than they use other methods. This practice results from long-term use of reducing sugar tests to detect stickiness in immature cotton, the recent certification (1993) of the SCT as the international standard test for insect-related stickiness, and the relatively high cost of the SCT.

Reducing sugar tests are calibrated using the most abundant monosaccharide in immature fiber, glucose, as the standard reductant. Several of the major component-sugars in aphid (Aphis spp.) and whitefly (Bemisia spp.) honeydews are non-reducing sugars. The specific reactions of others, such as trehalulose, the principal component of whitefly honeydew, have not been reported for reducing-sugar tests.

This report provides quantitative, reducing-sugar test-values for the major insect sugars found on contaminated lint. These data illustrate some of the limitations of applying reducing sugar tests for estimating stickiness caused by insects. The major aphid and whitefly honeydew sugars, melezitose, trehalulose, and sucrose, do not yield values comparable to glucose in reducing-sugar tests. Empirical correlations of reducing sugar test data and stickiness potential may be possible, if the approximate composition of the contaminants is known. However, use of criteria for stickiness based on absolute levels of glucose reducing equivalents will probably underestimate stickiness, if the primary source of contamination is from insect sugars.

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Introduction

Stickiness in lint is a serious quality problem for the cotton industry, a burdensome processing problem for the textile industry, and a source of major economic loss for both (Hector and Hodkinson, 1989). Stickiness may occur due to an elevated concentration of constituent plant sugars or from contamination by honeydew sugars produced by cotton aphids (Aphis gossypii Glover) or whiteflies in the Old World (Bemisia tabaci Gennadius) and in the New World (Bemisia argentifolii Bellows & Perring). Over the past decade, stickiness has been reported with increasing frequency (ITMF 1993, 1995, 1997). The increase in honeydew contamination is coincident with the expansion in range and severity of Bemisia spp. throughout the pantropic and warm temperate regions (Campbell et al. 1995). In one or more recent years, lint stickiness has been reported in cottons from Africa, the Middle East, Pakistan, India, and the U.S. (ITMF, 1993, 1995, 1997).

Reducing-sugar tests have been used by textile mills to screen cottons suspected of having high concentrations of plant sugars (Perkins, 1971; Perkins, 1993). Reducing sugar contents in lint as high as 0.6% in West Texas cotton and 0.3% in Israel cotton have been indicated as levels at which fiber processing difficulties might be anticipated (Elsner et al. 1983, TRC, 1988). However, it is clear that broad ranges of reducing sugar test levels may be observed in lots of cotton with no strong correlation with stickiness potential (Perkins, 1991). A physical test method, employing the Sticky Cotton Thermodetector (SCT) developed by the Centre de Cooperation International en Researche Agronomique pour le Developpement (CIRAD) is the International Textile Manufacturer's Federation (ITMF) standard method for estimating stickiness in cottons suspected of contamination by honeydew sugars (Frydrych, 1986; Brushwood, 1993).

Both aphid and whitefly honeydews are mixtures of several reducing sugars, non-reducing sugars, and certain other sugars, seldom found other than in honeydew (Byrne and Miller, 1990; Hendrix et al., 1991; Tarczynski et al., 1992). The principal components of aphid honeydew, collected immediately following deposition, are a trisaccharide, melezitose, and a disaccharide, sucrose, which are non-reducing sugars. The principal components of whitefly honeydew, also when freshly deposited, are a disaccharide, trehalulose, and melezitose and sucrose. Upon weathering, or with decomposition by micro-organisms, these honeydew sugars break down to monosaccharides. Therefore, we believe that honeydew contamination on lint could contain more monosaccharides than would be estimated based on the analysis of freshly collected honeydew (Perkins, personal communication).

The reducing reactions of melezitose and trehalulose are not fully described in technical literature. The research reported here was done to characterize the reducing potential of

individual aphid and whitefly honeydew sugars in selected reducing sugar tests differing in chemical procedures and the relative oxidizing strength of their principal oxidants.

Materials and Methods

Sugar Standards

Stock solutions of glucose, fructose, sucrose, melezitose, trehalulose, raffinose, and turanose were prepared for the range of concentrations specified for the four tests (Table 1). All sugars, except trehalulose, were purchased from Sigma Chemical Company, St. Louis, MO. Trehaluose was obtained as a 58% w/w syrup from Mitsui Sugar Co., Kawasaki, Japan and purified to >99.8% by chromatography using an Amberlite CG-120 column (2.5 x 153 cm) with deionized water as the mobile phase. Turanose and raffinose were tested because of certain configurational similarities to honeydew sugars that could be relevant to the design of analytical procedures. Since they are not major honeydew components no further data on these sugars is presented here.

Reducing Sugar Tests

Sugar molecules are carbohydrates comprised of five or six-membered rings. The rings may occur singly or in polymers of two to several units. The primary functional (reactive) sites on the individual units (rings) are carbonyl or hydroxyl groups. The simple sugars are termed aldoses, if the carbonyl groups are formed from a terminal carbon; and ketoses, if the carbonyl groups contain a non-terminal carbon. Sugars are relatively stable in mild acidic solutions, but salts of many heavy metals are reduced by solutions of certain alkaline sugars. Fehling's reagent, cupric hydroxide, complexed with tartrate, is often used as a step in determining the structure of sugars. Sugars that reduce Fehling's, and similar, reagents are termed reducing sugars. Both aldoses and ketoses reduce Fehling's solution, but ketones do not.

The reducing sugar reaction can also be used to quantify the carbohydrates oxidized by alkaline metal salts. Although the reaction may involve oxidation of adjacent ring-hydroxyls, as well as aldehyde groups, the net reaction has successfully been adopted for the empirical estimation of reducing sugars, and related reducing substances, by standardizing reaction conditions and by the use of appropriate calibration standards. Four reducing sugar methods, based on different chemical mechanisms, and different oxidation potentials were evaluated. All sugars were tested at from five to thirteen concentrations, as prescribed by the different methods, to establish respective concentration response curves. All tests were repeated. Means are presented graphically, and the data pooled among test-replications for computation of linear regressions. The bases of the four methods are described below. The reader is referred to the original papers for details of the procedures.

Sumner Test: (Marsh and Simpson, 1977) This method was initially developed for determining sugar in urine (Sumner, 1924). It is based on the reaction of dinitrosalicyic acid with water soluble reducing substances. Samples are incubated in a boiling water bath in the presence of alkaline 3,5-dinitrosalicyclate, and the developed color read by a spectrophotometer at 550 nanometers.

Nelson's Test: (Nelson, 1924; Somogyi, 1945) A copper reagent is prepared from sodium-hypophosphate, potassium-sodium tartrate, sodium hydroxide, copper sulfate, and sodium sulfate. The samples are boiled with the copper reagent, then reacted with an arseno-molybdate reagent, and read in a spectophotometer at 520 nanometers.

Folin's Test: (Rimon, 1982) This test is used in some laboratories in Israel and is a variation of the Benedict test (cupric hydroxide stabilized with citrate). The sample is boiled in a modified Fehling reagent, then cooled, reacted with a color developing reagent to form blue molybdenum oxide, and read in a spectrophotometer at 420 nanometers.

Perkin's Test: (Perkins, 1971) The test uses a strong oxidizing agent to react some sugars that would not be found with milder reagents. The sample is reacted with an excess of potassium ferricyanide in the presence of sodium carbonate. The reduced product, potassium ferrocyanide is determined by titration with standard ceric sulfate in acid solution in the presence of 0-phenanthroline ferrous sulfate (ferroin) as the end-point indicator.

Results and Discussion

The concentrations calculated for individual sugars, other than glucose, the standard, depends to some extent on the method used (Figure 1.). The principal difference, among the reducing sugar tests evaluated, is the relative strength of the oxidizing reagent. In general the stronger the oxidizing reaction the more complete the reaction, and the higher the computed sugar content.

A regression analysis was done to compare the relative rates of detection of sugars other than the standard, glucose, among the methods (Table 2). For each sugar a linear regression was found with the intercept set at x,y = 0. Highly significant linear regressions were found ($P < 0.001$). Within each method, the slope of the calibration curves for each individual sugar was divided by the slope found for glucose, thus comparing the relative recoveries in reducing equivalents. The reducing hexose, fructose served as a positive standard. Both the disaccharide, sucrose, and the trisaccharide, melezitose, are non-reducing sugars. As such they serve as negative standards, and their curves were found to be indistinguishable from $y = 0$. Trehalulose was estimated with from 36-87% efficiency according to the method, and the relative strength of the oxidation system (Table 2).

These results indicate problems with the direct application of reducing sugar tests for estimating stickiness potential in insect contaminated lint. Aphid honeydew contains approximately 38% melezitose and 12% sucrose (Hendrix et al., 1991). Neither sugar would be detected by a reducing sugar test. Whitefly honeydew contains approximately 44% trehalulose, 17%, melezitose, and 16% sucrose (Hendrix et al., 1991). Clearly the sucrose and melezitose would not be found, and the trehalulose would be underestimated to a greater or lesser extent depending on the method used.

Moreover, the inherent stickiness potential of the sugars varies (Miller et al. 1994). Mini-card ratings of 0,1,2,and 3 correspond with descriptions of stickiness potential as follows: no stickiness, slightly sticky, moderately sticky, and very sticky cotton, respectively. Over-spraying solutions of sugars on clean, dry lint to produce samples with 1% glucose, melezitose, sucrose, and trehalulose, respectively, produced contaminated cottons that gave mini-card ratings of 1.25, 2.25, 2.70, and 3.00, respectively. Thus reducing sugar tests miss or underestimate several abundant insect sugars that are appreciably stickier than glucose.

A Possible Mill Scenario

Underestimation of stickiness potential from the use of reducing sugar tests could occur in a number of ways. For example, a mill purchases cotton from an area that subsequently is found to have produced some sticky cotton in the same marketing year. The mill prudently screens selected bales using a simple, inexpensive reducing sugar test, such as a Clinitest, to estimate stickiness potential. The mean reducing sugar content is found to be 0.3%. If the problem is plant sugars, these bales are safe to run, with good humidity control and/or conservative blending of lay-downs. If the problem is actually aphid or whitefly contamination, it is probable that the stickiness potential has been underestimated on two counts: 1. the insect sugars have been underestimated, and 2. the sugars that were not properly quantified are inherently stickier than glucose. A third problem, outside the scope of this work, is that the insect sugars are deposited as discrete spots and readily adhere to working surfaces, whereas plant sugars are more evenly distributed throughout the fiber and do not lead to the rapid appearance of sticky spots on processing equipment.

Conclusions

Consistency in the respective compositions of honeydews from aphids and whiteflies feeding on cotton may provide empirical correlations between reducing sugar determinations and the levels of honeydew contamination on lint (Brushwood, in review). However, stoichiometric determinations of the absolute sugar contents of mixtures of reducing, non-reducing, and weak reducing sugars, such as are found in insect honeydews, are not possible using common reducing sugar methods. A possibility, not addressed by these data, is that false positives may occur with the use of narrowly targeted test procedures for diagnosis of complex mixtures such as insect honeydews.

Quantitative criteria for predicting stickiness, based on levels of reducing sugars found from testing plant sugars, can not be used alone to estimate the stickiness potential from honeydew sugars. Rather, quantification of the reducing equivalents of the major, individual sugars found in aphid and whitefly honeydew shows that common reducing sugar tests will underestimate the sugar content and stickiness potential of honeydew contaminated cotton, if the results are interpreted using information based on the stickiness potential found with chiefly plant sugars.

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Table 1. Properties of common honeydew sugars.

Sugar	Type	Molecular Weight	Reaction
glucose	monosaccharide	180.2	reducing
fructose	monosaccharide	180.2	reducing
sucrose	disaccharide	342.3	non-reducing
trehalulose	disaccharide	342.3	variably reducing
melezitose	trisaccharide	504.4	non-reducing

Table 2. Efficiency of trehalulose quantification by four reducing sugar methods, based on linear regression analysis.

Sugar	regression F-test	correlation r2	slope Beta	efficiency - % -
	---------- Folin test ----------			
glucose	4191 ***	0.998	0.00421	100
fructose	1796 ***	0.997	0.00356	84 +/- 4
trehalulose	969 ***	0.993	0.00152	36 +/- 5
	---------- Nelson test ----------			
glucose	543 ***	0.980	0.0098	100
fructose	749 ***	0.986	0.0877	89 +/- 4
trehalulose	545 ***	0.980	0.0466	47 +/- 4
	---------- Sumner test ----------			
glucose	6644 ***	0.999	0.529	100
fructose	9184 ***	0.999	0.522	99 +/- 1
trehalulose	15395 ***	0.999	0.392	74 +/- 1
	---------- Perkins test ----------			
glucose	784 ***	0.996	6.62	100
fructose	700 ***	0.994	6.68	101 +/- 4
trehalulose	689 ***	0.994	5.80	87 +/- 4

*** indicates significance at the P > 0.001 level.

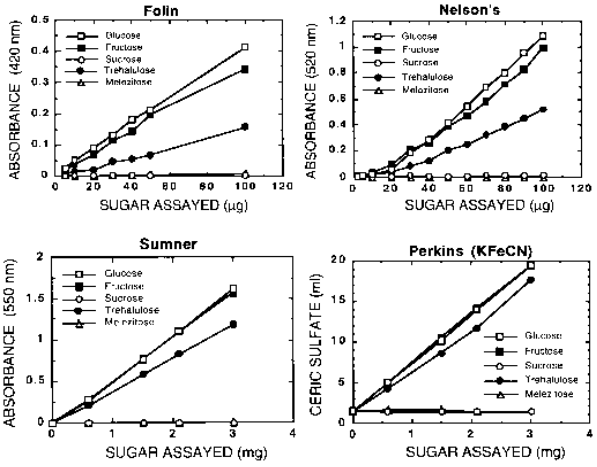

Figure 1. Estimation of the principal carbohydrate components of aphid and whitefly honeydew by four reducing sugar methods.