

## PHYSIOLOGY

### Modification of the Potassium Ferricyanide Reducing Sugar Test for Sugars from Extracts of Cotton Fiber

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#### INTERPRETIVE SUMMARY

The potential of cotton lint to stick to processing mill equipment is related to the presence and amount of sugars from physiological and/or insect sources. If levels of physiological sugars are high or significant amounts of insect honeydew contamination are present, lint stickiness can severely limit processing efficiency. As a quality control measure, assays such as the potassium ferricyanide ( $K_3Fe[CN]_6$ ) sugar test, have been developed to measure sugar levels on cotton lint. This test is relatively insensitive to two sugars, sucrose and melezitose, which are known to contribute to lint stickiness. These two sugars can be rendered detectable by the addition of a simple acid-hydrolysis step to the standard potassium ferricyanide sugar test. Cotton producers, brokers, and processors at textile mills who use this modified sugar test during marketing and processing can measure sugar content quantitatively, and thereby enhance their confidence in predicting cotton stickiness potential and lint processing efficiency.

#### ABSTRACT

For many years the potassium ferricyanide ( $K_3Fe[CN]_6$ ) standard sugar test—also known as the Perkins test—has been used by the textile industry to quantify the content of sticky sugars on cotton (*Gossypium hirsutum* L.) lint. This test, however, is a reducing sugar test and does not detect non-reducing sugars, which are known to contribute to the stickiness potential of the lint. Hence, poor correlations are often found between potassium ferricyanide sugar-test results and physical stickiness ratings, such as sticky-cotton thermodetector and

minicard measurements. This lack of detection is particularly true of cotton lint contaminated with aphid (*Aphis* spp.) honeydew. This study was designed to determine whether some of the non-reducing sugars extracted from cotton lint could be converted to reducing sugars prior to the potassium ferricyanide sugar test. Treatment with a mild (0.2 M) sulfuric acid solution converted sucrose (a physiological sugar) and melezitose (an insect-honeydew sugar) to reducing sugars that are detectable by the standard potassium ferricyanide test. Complete conversion of these sugars was verified by use of high performance liquid chromatography (HPLC). The modified potassium ferricyanide sugar test provided a more realistic quantification of sugars present, and the difference between standard and modified sugars was directly proportional to the amount of sucrose and melezitose present. Our modified potassium ferricyanide test did not substantially enhance the correlation between measured sugar content and predicted sticky-cotton-thermodetector stickiness potential for non-honeydew and whitefly-honeydew (*Bemesia* spp.) contaminated cotton. But, correlations between sugar content and sticky-cotton-thermodetector stickiness for cotton lint contaminated by aphid-honeydew were improved by at least 20%.

Since being introduced in the early 1970s, the potassium ferricyanide or Perkins test (Perkins, 1971) has been used extensively in the cotton industry to determine levels of reducing sugars present on cotton lint. The test is popular due to its relative simplicity and low cost. It is reliable, requires initial investments of less than \$500 (including chemicals and a small hot plate), and can be set up easily. All necessary chemicals are commercially available and have a long shelf life. Through the years this test has been used by the textile industry as an accurate and reproducible

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**Abbreviations:** HPLC, high-performance liquid chromatography.

method for successfully screening mixes and laydowns for potential sticky-lint problems.

The Perkins test determines the amount of reducing material in water-surfactant extracts of cotton lint by comparing the reducing ability of these extracts to that of glucose as the reducing-sugar standard. An excess of potassium ferricyanide ( $K_3Fe[CN]_6$ ) in the presence of sodium carbonate ( $Na_2CO_3$ ) oxidizes reducing material (principally sugars) in the extract and, in turn, is reduced to potassium ferrocyanide ( $K_4Fe[CN]_6$ ). Titration with ceric sulfate [ $Ce(SO_4)_2$ ] in acid solution in the presence of an *o*-phenanthroline ferrous sulfate indicator determines the amount of potassium ferrocyanide present, as the potassium ferrocyanide level is proportional to the amount of reducing substances present in the untreated extract.

Positive relationships have been found between potassium ferricyanide test sugar determinations and stickiness as measured by the sticky cotton thermodetector and minicard cotton stickiness tests (Brushwood and Perkins, 1993; Brushwood, 1998). However, good correlations between test methods were found for non-honeydew and whitefly-honeydew contaminated cottons only because acceptable correlations depend on reducing sugars being the predominant carbohydrates present. Extracts from cotton lint rated as uncontaminated, i.e., non-honeydew, generally contain large amounts of glucose, fructose, and sucrose, totaling between 700 and 850 g kg<sup>-1</sup> (70-85% by weight) of the sugars present identified and quantified by HPLC. Combined contents of glucose and fructose (both reducing sugars) usually range from 500 to 650 g kg<sup>-1</sup>. The content of sucrose (a non-reducing sugar) may range from trace amounts up to 200 g kg<sup>-1</sup>, depending on cotton genotype and environmental conditions during fiber production. The reducing sugars trehalulose, fructose, and glucose, followed in order of concentration by melezitose and sucrose (non-reducing sugars), turanose (a reducing sugar), unidentified mono- and disaccharides, and oligosaccharides predominate in extracts from cotton lint contaminated with whitefly honeydew (Byrne and Miller, 1990; Hendrix et al., 1992; Tarczynski et al., 1992). About 700 g kg<sup>-1</sup> of identified sugars in whitefly-honeydew are reducing sugars. The sugars trehalulose, melezitose, and turanose are found exclusively in insect honeydew. Trehalulose

concentrations in cotton lint heavily contaminated with whitefly honeydew can exceed 350 g kg<sup>-1</sup> of total sugar extracted, and the ratio of trehalulose to melezitose is usually about 2 to 1 (Brushwood, 1998).

Cotton lint contaminated with aphid honeydew contains larger amounts of unidentified and non-reducing sugars than does lint contaminated with whitefly honeydew. Melezitose concentrations can exceed 200 g kg<sup>-1</sup> of total identified sugar content in aphid honeydew, but there is usually little or no trehalulose. Laboratory HPLC analyses have determined that the combined concentrations of the non-reducing sugars sucrose and melezitose can be as high as 350 to 400 g kg<sup>-1</sup> of total identified sugars (Brushwood and Perkins, 1994; Miller et al., 1994).

Generally, poor correlations have been found between potassium ferricyanide sugar test results and sticky ratings determined by sticky cotton thermodetector or minicard in tests of lint contaminated by aphid honeydew.

Because the non-reducing sugars sucrose (present in both uncontaminated and honeydew-contaminated lint) and melezitose (found in honeydew-contaminated lint) are present and have considerable influence on overall lint stickiness, this paper proposes a simple modification of the classic potassium ferricyanide reducing sugar test. The change is to acid-hydrolyze the extracts prior to titration so that sucrose and melezitose are converted to reducing sugars. After hydrolysis, these sugars would be included in the total sugar content to give a more realistic value of sugar content, thereby enhancing the prediction of processing stickiness through use of a simple chemical test method, rather than a complex, expensive physical test requiring additional instrumentation.

## MATERIALS AND METHODS

### Total Sugar Analysis

Water-surfactant extracts from cotton lint samples and specific sugar mixtures were analyzed as specified in the potassium ferricyanide reducing-sugar test (Perkins, 1971) and by the modified sugar test to be described here. The modified test followed the same procedure as the original potassium ferricyanide test with one exception. When the

extract aliquot to be analyzed was added to the 125-mL Erlenmeyer flask, 10 mL of 0.2 M H<sub>2</sub>SO<sub>4</sub> were also added. The extract-acid solution was then boiled on a hot plate for 2 min. This step converted any sucrose and melezitose that might have been present to reducing sugars. After the solution was cooled to room temperature, 10 mL of 0.4 M NaOH were added to neutralize the acid in the flask. From this point, the procedure for the original test was followed, including the reboiling step after the addition of the potassium ferricyanide/sodium carbonate solution. A minimum of two determinations using both the standard and hydrolyzed-sugar tests were made on all lint extracts. Data presented in this study are means of these determinations.

### Individual Sugar Measurements

Where applicable, anion HPLC was used (Brushwood and Perkins, 1994) to identify and determine concentrations of sugars in extracts from cotton lint samples. The HPLC analyses were performed with a Dionex DX 300<sup>1</sup> spectrophotometer (Dionex, Sunnyvale, CA) equipped with a PA-1 carbohydrate separation column and column guard. The eluant was 200 mM NaOH in isocratic mode fed at a rate of 1.0 mL min<sup>-1</sup> with a column operating pressure ranging from 9.7 H 10<sup>6</sup> to 11.0 H 10<sup>6</sup> Pa. The solvent for all sugars, sugar standards, and cotton fiber extracts was distilled water. In addition to verification of the acid hydrolysis of sucrose and melezitose to reducing sugars, concentrations of other sugars present before and after acid hydrolysis also were determined. Sugar concentration calculations were based on comparisons to sugar standards prepared and run concurrently under the same assay conditions.

### Fiber Stickiness Measurements

Stickiness ratings for each cotton lint sample (a minimum of two measurements per sample) were

conducted using a manual thermodetector stickiness tester (SCT, IRCT, Montpellier, France) operating under vendor-recommended conditions (Brushwood and Perkins, 1993). The following rating levels for stickiness of raw cotton lint were used: 0 to 4 visible sticky spots = nonsticky, 5 to 14 spots = slightly sticky, 15 to 24 spots = moderately sticky, and more than 24 spots = extremely sticky. The baseline reference was 6 to 8 sticky spots. As a rule, cottons with sticky-cotton-thermodetector sticky spot counts in excess of eight were considered to have potential for sticking during textile processing.

### Analyses of Extracts from Field-Grown Lint

We selected four non-honeydew contaminated lint samples from three U.S. growing regions. Ten lint samples contaminated with whitefly honeydew were obtained from New Mexico, and nine line samples contaminated with aphid honeydew were procured from various U.S. locations. Each sample was characterized in duplicate by HPLC analyses for content of individual sugars and rated for stickiness potential using the sticky cotton thermodetector as described above. Sugar determinations by the standard and modified potassium ferricyanide tests were then conducted.

## RESULTS AND DISCUSSION

### Selection of Acid Concentration for Conversion

For years the potassium-ferricyanide reducing-sugar test has been widely used by the textile industry. This modification was designed to convert specific non-reducing sugars to reducing states without introducing major changes in the original procedure. Sulfuric acid concentrations ranging from 0 to 2.0 M were used to hydrolyze non-reducing sugars found in extracts from cotton lint. Particular attention was given to acid effects on those sugars found on lint contaminated with insect honeydew.

All acid concentrations in the range between 0.2 and 0.5 M (assayed with a minimum of two replicates per sample at each acid concentration) were completely successful in reducing the sugars, sucrose, and melezitose, to their reducing states. Acid concentrations below 0.2 M did not completely convert sucrose and melezitose to reducing sugars.

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<sup>1</sup>Trade names are necessary for reporting factually on available data. The USDA neither guarantees nor warrants the standard of the product or service. By using this and other names the USDA implies no approval of the product or service to the exclusion of others that also may be suitable.

Two other non-reducing sugars identified in extracts are myoinositol and trehalose. Natural concentrations of the former can vary from 0 to 12% by weight in lint samples not contaminated with honeydew. Concentrations of trehalose generally do not exceed 20 g kg<sup>-1</sup> of total sugars. No hydrolysis of myoinositol to reducing sugars was detected in the acid range tested. It is possible that part or all of the trehalose was converted to reducing sugars at acid treatment concentrations above 0.3 M; but sugar assay results for non-honeydew, aphid-honeydew, and whitefly-honeydew extracts did not change quantitatively when subjected to acid hydrolysis at concentrations up to 0.5 M.

When the sulfuric acid concentration exceeded 0.5 M, slight discoloration of the glucose standard, some individual sugar solutions, and the fiber extracts occurred during the initial boiling process. As the treatment acid concentration increased above 0.5 M, discoloration also increased until all solutions were either black or greenish-black in color. Accurate visual titration end points were difficult or impossible to determine at acid treatment concentrations above 0.5 M. Therefore, 0.2 M sulfuric acid was selected as the assay concentration. This concentration was completely effective in converting sucrose and melezitose to their reducing states, had minimum effects on the other sugars present, and was practical from economic and safety standpoints.

### Sugar Calibration Results

Table 1 shows the calculated standard (non-hydrolyzed) and modified calibration efficiencies for six of the most prevalent sugars on insect-contaminated and uncontaminated cotton lint.

Efficiencies for both tests are compared with glucose as the reference standard. Triplicate calibrations were determined for each sugar at concentrations of 0, 1, 2, 3, 4, 5, or 6 g kg<sup>-1</sup> (0, 0.1, 0.2, 0.3, 0.4, 0.5 or 0.6% w/w) based on the weight of 1 g of extracted fiber. The amount of titration material (ceric sulfate) necessary for determining individual sugar concentrations increased when the modified test was used. Glucose, fructose, and turanose determination sensitivities increased between 70 and 80 g kg<sup>-1</sup>, and sensitivity to trehalulose rose about 45 g kg<sup>-1</sup>. Individually, efficiencies for fructose, turanose, and trehalulose were essentially the same when either sugar test was used. However, the standard error of determination for trehalulose in the modified test decreased in comparison to the standard sugar test because visual titration end points were easier to detect.

The most important consequence of the test modification was reflected in the sucrose and melezitose analyses. These sugars were converted to reducing sugars, making them detectable by the potassium ferricyanide sugar test. Sucrose and melezitose efficiency ratings went from 0 to 1020 g kg<sup>-1</sup> and 860 g kg<sup>-1</sup>, respectively. Collectively, total sugar detection efficiencies for the six sugars assayed increased by an average of 630 to 940 g kg<sup>-1</sup>. In general, efficiency decreased as sugar molecular weight increased.

### Effect of Hydrolysis on Individual and Mixed Sugar Compositions

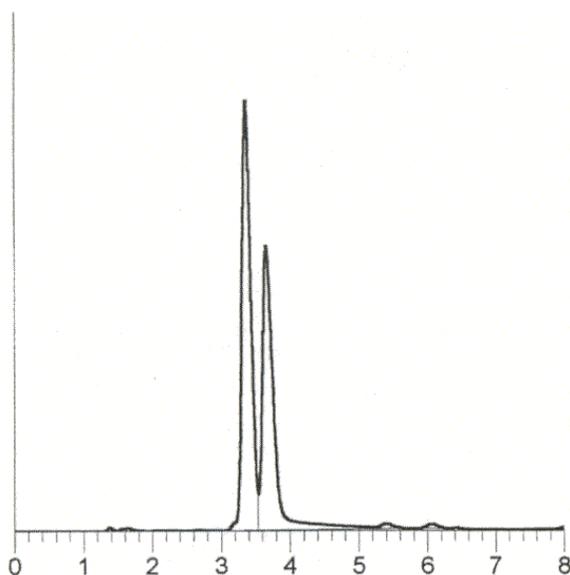
Figures 1 and 2 are chromatograms representing the acid-hydrolyzed sugars sucrose and melezitose. In each case, the initial sugar concentration before treatment was 6 g kg<sup>-1</sup> or 0.6% w/w, based on the

**Table 1. Detection efficiency of the standard and modified potassium ferricyanide tests for six sugars commonly found on cotton lint (sugar standards: 0-6 mg g<sup>-1</sup> fiber).**

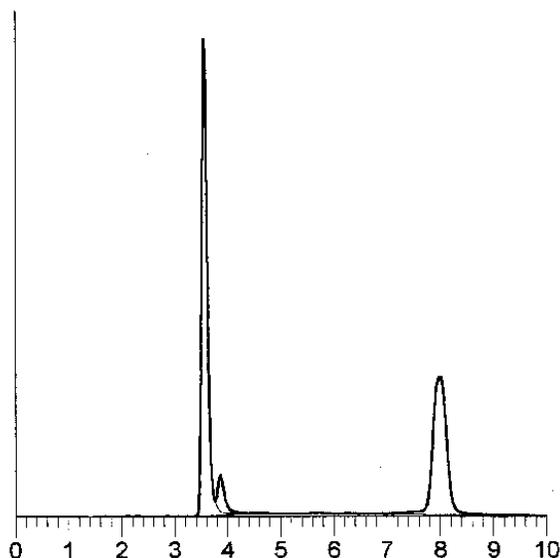
Sugar	Molecular Weight	Type	Standard sugar test		Modified sugar test	
			Slope <sup>†</sup>	Efficiency <sup>‡</sup>	Slope	Efficiency
				%		%
Glucose	180.2	Reducing	5.99	100	6.42	100
Fructose	180.2	Reducing	6.19	103 ± 4	6.68	104 ± 5
Sucrose	342.3	Non-reducing	0	0	6.57	102 ± 2
Turanose	342.3	Reducing	4.97	83 ± 1	5.35	83 ± 3
Trehalulose	342.3	Reducing	5.53	92 ± 7	5.77	90 ± 2
Melezitose	504.4	Non-reducing	0	0	5.55	86 ± 6

<sup>†</sup> mL ceric sulfate / mg sugar.

<sup>‡</sup> Efficiency = slope of sugar detection gradient/slope of glucose detection gradient x 100.



**Fig. 1.** An HPLC chromatogram of sucrose after acid hydrolysis with 0.2 M sulfuric acid shows peaks at 3.4 and 3.8 min, indicating the reducing sugars glucose and fructose, respectively.



**Fig. 2.** An HPLC chromatogram of melezitose after acid hydrolysis with 0.2 M sulfuric acid shows peaks at 3.4, 3.8, and 8.0 min, indicating the reducing sugars glucose, fructose, and turanose, respectively.

amount of sugar extracted from 1.0 g of fiber. Sucrose (Fig. 1), which normally has a retention time of 7.2 min, was converted to 505 g kg<sup>-1</sup> glucose with a retention time of 3.4 min and 495 g kg<sup>-1</sup> fructose with a peak at 3.8 min. Melezitose (Fig. 2), which

normally has a retention time of 11.2 min, was converted to 610 g kg<sup>-1</sup> glucose, 260 g kg<sup>-1</sup> turanose, with a retention time of 8.0 min, and 130 g kg<sup>-1</sup> fructose. Hydrolyzed turanose (using the same acid concentration) was converted to 70 g kg<sup>-1</sup> glucose, and 30 g kg<sup>-1</sup> fructose with 900 g kg<sup>-1</sup> remaining as turanose. The trehalulose concentration, after hydrolysis, remained basically the same since only about 20 g kg<sup>-1</sup> were converted to equal amounts of glucose and fructose. Glucose and fructose levels, as determined by HPLC, were unchanged by the 0.2 M acid treatment.

A mixture containing 1.0 mg each of the six sugars listed in Table 1 (a total sugar concentration of 6 g kg<sup>-1</sup>) was prepared and tested by the standard and modified potassium ferricyanide tests. The average efficiency rate (three replicates) for the standard sugar test was 670 g kg<sup>-1</sup> recovery. This rate agrees very well with a theoretical average of 630 g kg<sup>-1</sup> for the individual sugar calibrations in Table 1. Three replicates of hydrolyzed versions (modified sugar test) of the same sugar mixture averaged an efficiency rate of 940 g kg<sup>-1</sup> recovery, an increase of 400 g kg<sup>-1</sup>. This result also agrees with the means in Table 1 and demonstrates, using mixtures of sugars, that the modified potassium ferricyanide test is successful in converting sucrose and melezitose to reducing sugars.

### Analysis of Cotton Lint Extracts

We selected four uncontaminated lint samples from three U.S. growing areas (samples 1-4 in Table 2). Samples 5-14 in Table 2 represent 10 New Mexico lint samples that were contaminated with whitefly honeydew. Samples 15-23 are from various U.S. locations and indicate nine raw lint samples that were contaminated with aphid honeydew. Sugar determinations (at least two duplicates for each of the 23 samples) were conducted by the standard and modified potassium ferricyanide tests (Table 2). Each sample was assayed in duplicate by HPLC for individual sugar content and rated by sticky cotton thermodetector (also in duplicate) for stickiness potential. Test result means, including sucrose and melezitose concentrations measured by HPLC, are shown in Table 2. Concentrations of sugars found by the modified potassium ferricyanide test reflect the amounts of reducing sugars initially present plus the

**Table 2. Standard and modified potassium ferricyanide sugars, individual sucrose and melezitose concentrations, and sticky-cotton thermodetector (SCT)-stickiness ratings for non- and honeydew contaminated cottons.**

Sample ID	SCT‡ Rating	Sugar Content†			
		Standard Test Mean ± standard error	Modified Test Mean ± standard error	Sucrose	Melezitose
No. -----g kg <sup>-1</sup> -----					
<b>Non honeydew lint samples</b>					
1	2	0.80 ± 0.05	1.10 ± 0.05	0.12	0
2	3	1.30 ± 0.15	1.90 ± 0.05	0.17	0
3	5	1.40 ± 0.05	1.60 ± 0.10	0.08	0
4	5	2.00 ± 0.05	2.20 ± 0.05	0.07	0
<b>Whitefly-honeydew contaminated lint samples</b>					
5	6	2.40 ± 0.05	3.40 ± 0.10	0.10	0.05
6	7	3.10 ± 0.50	4.40 ± 0.05	0.19	0.03
7	8	3.50 ± 0.20	4.10 ± 0.05	0.14	0.04
8	18	12.10 ± 0.40	14.30 ± 0.35	0.48	1.94
9	19	12.10 ± 0.05	16.00 ± 0.30	0.24	2.06
10	19	12.7 ± 0.60	17.40 ± 0.50	0.25	0.29
11	17	13.10 ± 0.20	16.80 ± 0.15	0.26	2.23
12	15	13.40 ± 1.90	18.00 ± 0.15	0.40	2.68
13	21	14.40 ± 2.05	20.50 ± 0.50	0.58	2.74
14	16	14.50 ± 0.20	18.60 ± 0.20	0.27	2.90
<b>Aphid-honeydew contaminated lint samples</b>					
15	22	2.70 ± 0.05	3.60 ± 0.05	0.06	0.22
16	8	2.80 ± 0.05	3.10 ± 0.05	0.06	0.10
17	18	2.90 ± 0.05	4.10 ± 0.05	0.07	0.23
18	9	3.50 ± 0.05	4.30 ± 0.20	0.07	0.16
19	32	4.10 ± 0.10	4.90 ± 0.10	0.07	0.23
20	23	4.60 ± 0.05	6.00 ± 0.30	0.13	0.13
21	96	5.70 ± 0.10	11.90 ± 0.90	0.22	0.40
22	66	5.80 ± 0.10	10.00 ± 1.00	0.66	0.31
23	100	5.80 ± 0.20	10.60 ± 0.60	0.27	0.33

† Mean of two determinations per sample.

‡ Mean number of sticky spots counted on sticky-cotton thermodetector.

reducing sugars produced by the acid hydrolysis step.

### Non-Insect and Whitefly-Honeydew Contaminated Lint

Differences between standard and modified sugar levels for the non-honeydew lint samples (samples 1-4) were related to the amount of sucrose present. The 10 whitefly-honeydew contaminated cottons (samples 5-14) were selected to represent honeydew contamination ranging from low to very high. Without hydrolysis, samples 8-14 had high sugar contents in excess of 12 g kg<sup>-1</sup> and high sticky-cotton-thermodetector stickiness ratings (moderate to extremely sticky range) because each contained concentrations of the reducing sugar trehalulose in excess of 300 g kg<sup>-1</sup>. Melezitose concentrations in each of these samples ranged from 160 to 200 g kg<sup>-1</sup>. The stickiness levels in these particular cottons

reflect the presence of the very sticky honeydew sugars, trehalulose and melezitose, which account for more than 500 g kg<sup>-1</sup> of the identified sugars.

Figures 3a and 3b are chromatograms of a typical extract from whitefly-contaminated cotton before and after hydrolysis with acid. After conversion (Fig. 3b), the concentrations of glucose (3.4-min peak) fructose (3.8-min peak), and turanose (8.0-min peak) increased. Melezitose (11.2-min peak) and sucrose (7.2-min peak), which initially represented 190 and 40 g kg<sup>-1</sup> of total sugars, were no longer present. The hydrolysis step had minimal effect on other routinely determined sugars, including two non-reducing sugars, myoinositol and trehalose, which appear as peaks at 1.8 and 2.8 min, respectively. After hydrolyzing some major non-reducing sugars before titration, the modified test sugar content for this lint extract was 420 g kg<sup>-1</sup> greater than the value obtained from the standard potassium ferricyanide test. This result agrees with

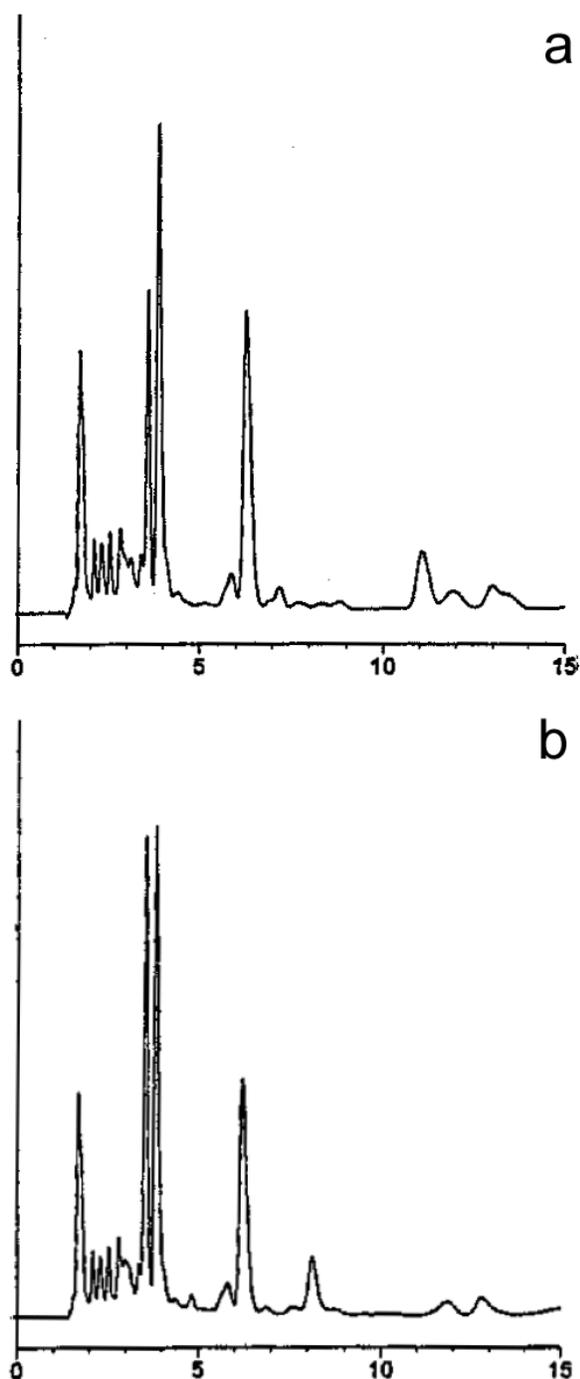


Fig. 3. (a) An HPLC chromatogram of cotton extract prior to acid hydrolysis and (b) an HPLC chromatogram of the same material as in 3a after hydrolysis with 0.2 M sulfuric acid, showing the complete conversion of melezitose that peaked at 11.2 min and sucrose that peaked at 7.2 min to glucose, fructose, and turanose (3.4, 3.8 and 8.0 minutes, respectively).

those obtained using the mixture of pure sugars. The correlation coefficient ( $r^2$ ) between differences in standard and modified test sugar levels and combined

melezitose and sucrose content determined by HPLC analyses for the whitefly-honeydew contaminated and non-insect cottons (samples 1-14) was 0.90.

Correlation coefficients for the relationship between measured sugar content and sticky-cotton-thermodetector stickiness counts (samples 1-14) were exactly the same (0.93) for the standard and modified potassium ferricyanide tests. Using a 6 to 8 sticky spot sticky-cotton-thermodetector measurement as a stickiness threshold, measured sugar concentrations above  $3.5 \text{ g kg}^{-1}$  based on fiber weight for the standard potassium ferricyanide test would represent the point at which concerns about fiber processing problems might occur. The analogous thresholds set with the modified test would be approximately  $4.5 \text{ g kg}^{-1}$ .

#### Lint Contaminated with Aphid Honeydew

When equivalent sugar tests were performed, stickiness ratings of cotton lint contaminated with aphid-honeydew (samples 15-23) were much higher than ratings for whitefly-contaminated lint samples. Five lint samples contaminated with aphid honeydew were rated as extremely sticky ( $>24$  sticky spots) when the standard potassium ferricyanide sugar assay content was less than  $5 \text{ g kg}^{-1}$ . Seven lint samples contaminated by whitefly-honeydew were rated as moderate to extremely sticky but averaged  $13.2 \text{ g kg}^{-1}$  sugar content in the same unmodified test. Average total sucrose and melezitose concentrations per sample were also about five times lower for aphid-honeydew samples than for lint samples contaminated with whitefly-honeydew. Trehalulose levels from the aphid-honeydew contaminated lint averaged less than  $20 \text{ g kg}^{-1}$  of total identified sugars. The correlation coefficient ( $r^2$ ) for the relationship between differences in the two sugar tests (standard and modified) and total sucrose and melezitose concentrations was 0.66. This is much lower than the 0.90 determined for whitefly-honeydew contaminated cottons.

The correlation coefficient ( $r^2$ ) values for the relationship between sticky-cotton-thermodetector stickiness counts and the two sugar tests were 0.74 for the standard test and 0.91 for the modified potassium ferricyanide test. Thus, the modified sugar test improved the sugar/stickiness prediction relationship by approximately 23% on a weight

basis. Stickiness thresholds for the two sugar tests with aphid-honeydew contaminated lint (using the same criteria of 6-8 sticky spots) were around 2.8 g kg<sup>-1</sup> for the standard test and 3.1 g kg<sup>-1</sup> for the modified potassium ferricyanide tests. In this study, much of the stickiness associated with aphid-honeydew contamination was due to non-reducing material that was not identified.

### CONCLUSIONS

A simple acid-hydrolysis step added to the sample preparation phase of the standard potassium ferricyanide sugar test converted the sugars sucrose and melezitose, which are normally non-reducing, to reducing sugars detectable by the assay. Mild acid hydrolysis also enhanced the clarity and accuracy of visual end-point in the assay titration. Differences between lint-extract sugar concentrations determined by the standard and modified potassium ferricyanide tests were directly proportional to the sucrose and melezitose contents. Sugar levels determined by either the standard or modified sugar test were excellent predictors of sticky-cotton-thermodetector-measured stickiness potential for non-honeydew and whitefly-honeydew contaminated cottons ( $r^2 = 0.93$ ). Aphid-honeydew contaminated cottons were rated much stickier than whitefly-honeydew contaminated cottons. When the modified potassium ferricyanide test was used, correlations between sticky-cotton-thermodetector sticky spot counts and sugar concentration determinations improved from 0.74 to 0.91.

In the standard evaluation and screening of raw cotton lint, the potassium ferricyanide sugar tests (standard or modified) are excellent and valuable predictors of cotton stickiness on non-honeydew and whitefly-honeydew contaminated cottons. Predictions of stickiness potential by chemical sugar analyses for aphid-honeydew contaminated cottons, although not as precise as with whitefly-honeydew contaminated cottons, were definitely improved by the use of the modified potassium ferricyanide sugar test.

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