# CHARACTERISTICS OF ENTOMOLOGICAL SUGARS APPLIED TO THE SURFACE OF RAW COTTON

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## Abstract

A single non-insect contaminated cotton was treated with different concentrations of two sugars identified as unique to insect honeydew. Trehalulose (a disaccharide), the most predominant sugar found on heavily contaminated whitefly cottons, and melezitose (a trisaccharide), found on both whitefly and aphid contaminated cottons, contribute substantially to the stickiness potential of cotton lint. High performance liquid chromatography (HPLC) analysis of extracts from these cottons to identify and quantitate individual carbohydrate concentrations, chemical sugar analysis to determine reducing sugars present, Thermodetector (TD) stickiness measurements, near infrared (NIR) spectra scans, and differential moisture meter measurements were run to characterize untreated and treated cottons that were conditioned to four different fiber moisture levels. Statistical analysis using chemical analysis and NIR spectra data resulted in the selection of twelve wavelengths and the fiber moisture content as independent variables in multiple regression equations to predict concentrations of entomological sugars on these cottons. Poor predictability was obtained looking at the overall range of fiber moistures from 4.6 to 9.3%. When the calculations were divided into specific moisture ranges, linear correlation coefficients of predictability increased significantly. A discriminant analysis procedure was able to classify cotton samples into two classes of the entomological sugar contents with 89.2% success ratio.

## Introduction

Insect honeydew contamination from aphids and whiteflies on raw cottons has greatly influenced cotton production worldwide for a number of years (Wyatt, 1976; Hector and Hodkinson, 1989; Perkins, 1991). Stickiness in the form of highly concentrated randomly deposited droplets from the above insects can affect cotton quality in ginning and in the textile mill, sometimes making processing virtually impossible. Heavily sticky lint deposits can build up on machinery making processing virtually impossible. These sticky honeydew materials have been isolated and identified as containing in addition to the normal plant sugars, highly complex carbohydrates that contribute significantly to lint stickiness. Two sugars identified as unique to aphid and whitefly honeydew are trehalulose and melezitose (Byrne and Miller, 1990; Hendrix, et. al., 1992; Tarczynski, et. al., 1992; Hendrix, et. al., 1993). Whitefly honeydew contaminated cotton sugar extracts generally contain 1.5 to 2 times more of the disaccharide trehalulose than the trisaccharide melezitose (Brushwood, 1998). Aphid honeydew contaminated lint extracts have been determined to also have melezitose present, little or no trehalulose, and proportionately larger aggregates of unidentified oligosaccharides.

Sophisticated analytical techniques such as mass spectroscopy (MS), gas chromatography (GC), and more recently high performance liquid chromatography (HPLC) have been very successful in identifying and characterizing individual sugars and other components in extracts from cottons and honeydew (Brushwood and Perkins, 1994). Highly positive relationships between certain sugar levels and routine chemical sugar analysis and physical stickiness tests such as the standard thermodetector and subjective minicard procedure have been established (Brushwood and Perkins, 1993; Perkins, 1993). Two automated cotton stickiness testers, the Fiber Contamination Tester (FCT) and the High Speed Thermo-detector (H2SD) are also being evaluated for possible use. These tests, although good predictors of stickiness, are time consuming, require special sampling techniques and equipment, and are difficult to integrate into a protocol that requires rapid classing. Non-destructive, reliable, and rapid tests to identify potentially sticky cottons would be valuable screening tools in a fiber classing system. One such test would be on-line Near Infrared (NIR) analysis (Taylor, 1980; Taylor, 1988). Properly calibrated and corrected for micronaire and moisture differences, NIR has the potential to be a quick method to detect the presence of honeydew contamination or excessive levels of plant sugars on cotton.

The design of this study was to produce a set of cotton samples with known concentrations of entomological sugars present. By using NIR as a tool to measure concentrations of these sugars, a possible discriminant analysis procedure to classify honeydew contaminated cottons can be developed. The potential outcome of this study can be a precursor to a development of a simple device that can measure the entomological sugar levels on cotton using a limited number of optical filters at NIR wavelengths. Careful consideration was given to the use of a minimum number of NIR filters to achieve successful results. Another test, using differential resistance moisture measurements to identify honeydew and high sugars level on the surface of cottons also was investigated.

## Materials and Methods

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A single non-insect contaminated upland cotton provided by the Ginning Laboratory at Stoneville, MS was used in this study. By adding the honeydew sugars trehalulose and melezitose to this single lint, potential interferences from complex sugars usually found in natural honeydew and other sources were eliminated. Prepared samples were conditioned and subsequently analyzed by NIR at four different fiber moisture contents between 4 and 10% to determine the effects of moisture on these measurements.

After blending with five passes through a hopper blender (Syncromatic Blending System, Fiber Control Corp., Gastonia, NC) the clean lint samples were treated with pure entomological sugars. Predetermined amounts of trehalulose (Wako Chemical, Richmond, VA, USA) and melezitose (Sigma, St. Louis, MO,USA) were dissolved in distilled water and randomly applied in the form of small droplets on the surface of the cotton.

Ten gram fiber samples were evenly distributed in a mat form on approximately 500 cm square sheets of aluminum foil, then placed on a zeroed top loading balance. Using a 5-ml syringe with a 1.5 inch/21 gage needle, triplicates (30 grams) of 2, 4 and 6 mg/g (0.2, 0.4, and 0.6% based on fiber weight) of each sugar was added to the surface of the cotton. Stock solutions were prepared so that for each 10 g of fiber 1 g of water/sugar was added. Thus, a total of 10% moisture was added to achieve target concentrations of sugar on lint. The procedure was used for both sugars and a 60/40 mixture of trehalulose and melezitose. Total treated lint samples numbered 27 (triplicate 10 g samples of each treatment). After treatment, these samples and triplicate (10 g each) untreated samples were stored for two weeks at 21°C and 24% relative humidity to condition. Standard oven moisture contents for these samples were determined to be 4.7± 0.1% before blending with a rotary laboratory blender (Custom Scientific Instruments, Kearney, NJ) and finally by hand. The samples were conditioned again at 24% RH for five days before testing.

Individual sugar compositions of treated and untreated cottons (1 gram each) were determined by anion HPLC analysis using Dionex DX 300 and DX 500 Spectrophotometers. A routine high performance liquid chromatography procedure for analyzing (Brushwood and Perkins, 1994) aqueous extract from these cottons determined average individual carbohydrate concentrations (six replications/sample). Sugar standards of known concentrations were periodically run during analysis to correct for any possible variations in column and detector sensitivity. Individual sugar standard deviations by this method are normally less than 10% of the amount present. Reducing sugars (also 1 gram per sample) by the standard Perkins (Perkins, 1971) test were also determined for each treatment after the blending process. GRAF/IRCT Thermodetector (TD) stickiness tests (Brushwood, 1998) were determined on 2.5 gram manually prepared webs on all samples after each conditioning humidity. Stickiness ratings were averaged from triplicate measurements. Likewise, NIR spectrophotometer (Pacific Scientific. Model 6500) scans of each sample from 1100 to 2500 nm were conducted at each conditioning relative humidity. Two (2) gram lint samples (in triplicate) were read in four orientations. Thus, a total of 12 measurements per treatment. Moisture contents were determined on 1 gram samples by a routine oven moisture test. Wet cottons were dried overnight in a 105°C oven. Subsequent differences in wet and dry weight were used to calculate percent moisture. Corresponding portable moisture meter readings (meter provided by USDA, Stoneville, MS laboratory) were determined at the same time as oven moisture samples were selected. This small "bread box" size meter powered by a 12 volt DC system was reported to have a workable range of 5 to 9% when measuring cotton lint moistures.

We selected and tested all samples as specified at 24, 46, 70, and 81% controlled relative humidities, respectively, starting from the lower and progressing to the higher humidity at room temperature. In each case, a stabilizing period of at least one week in a temperature and humidity controlled room was allowed before commencing with fiber sample selection. Control (untreated) oven moistures were periodically determined during the conditioning periods to check on moisture levels.

Samples (2.5g each) for resistance moisture measurements at the Stoneville, MS laboratory were selected (30 total) at each relative humidity, sealed in individual zip-lock bags, labeled, and re-sealed in leakproof screw top jars.

## Results and Discussion

### Individual Sugar Concentrations

The plant sugars glucose, fructose, and sucrose in the untreated and treated extracts averaged 0.05, 0.04, and 0.01% (based on fiber weight), respectively. These three sugars represented 70 to 75% of the total HPLC sugars measured on the untreated cottons. Induced trehalulose, melezitose and the 60/40 mixtures of these two sugars were determined to be less than expected. Recovered materials averaged about 38% of the amount applied. Trehalulose treated cotton concentrations average 0.08, 0.14, and 0.17% for the low, medium, and high treatments, respectively (Table 1). These levels represent 40, 35, and 28% recoveries. Melezitose concentrations were determined to be 0.08, 0.15, and 0.18% or recoveries of 40, 38, and 30%. In combination, the 60/40 induced mixture cottons yielded trehalulose and melezitose concentrations of 0.05/0.04 (42%/50% recovery), 0.10/0.07 (42%/44% recovery), and 0.12/0.08% (33%/33% recovery). In all of the above determinations, melezitose retention rates

were slightly better than that of the trehalulose. Two factors may have contributed to lower than expected recoveries. First, the application technique using droplets from a syringe proved to be very inefficient. Secondly, we feel that preconditioning the cotton to a lower 4.7% moisture may have caused considerable loss of applied sugars, particularly during the rotary blending.

**Reducing Sugar Results**

Average Perkins reducing sugar concentrations for untreated and melezitose treated cottons were determined to be 0.24% (Table 1). Since melezitose is a non-reducing sugar, the standard Perkins sugar test does not detect melezitose. Perkins test values for the other samples reflect that trehalulose is a reducing sugar. Mean differences in reducing sugar content between untreated (control) and trehalulose treated cottons at the three treatment levels were determined to be higher than those calculated from HPLC analysis. Calculated reducing sugar concentrations were 0.10, 0.16, and 0.22% compared to 0.08, 0.14, and 0.17% for the low, medium, and high treatments, respectively. Conversely, mixed trehalulose/melezitose reducing sugar results predicted trehalulose concentrations to be equal to or slightly lower than calculated corresponding HPLC concentrations at 0.03, 0.10, and 0.12%. These results support the reliability and accuracy of our routine HPLC technique for analysis of the sugar trehalulose in extracts from insect contaminated cottons.

**Moisture Measurements**

Oven moisture contents from all four conditioning humidities were determined on each sample (Table 2). Fiber moisture contents at the lower humidity (24%) varied from an average low of 4.45% for the control (untreated) cotton to a high of 4.67% for the low melezitose content treatment. Overall average for these samples was 4.58 ± 0.07% at this humidity. At the other relative humidities, increasing from the lower to the higher ones of 46, 70, and 81% average fiber moisture contents were, 6.68 ± 0.07, 8.48 ± 0.06, and 9.33 ± 0.07%, respectively. Statistical analysis at a 99% confidence level did not indicate any significant difference in moisture content between untreated and treated samples at either level.

The portable moisture meter calibration with five preconditioned control cottons ranging from 5.4 to 8.6% yielded a simple coefficient of correlation R between conditioned moisture and meter reading of 0.97. Very good agreement was detected between meter reading and conditioned fiber moisture as determined by the oven method in the 5 to 7% range. Meter readings tended to drift higher at moisture content above 7%. The meter calibration was repeated twice, once before reading the 24%, and before the 70% relative humidity sample readings.

Five gram samples of each treated and untreated cotton were read on the portable meter at the 46 and 70% relative humidity conditions (Table 2). The sample was read, removed from the meter chamber, re-oriented, and read again. Resulting meter readings were the average of three readings. Meter readings were also attempted at the lower and higher humidities of 24 and 81%, however, the lower and higher limits of the meter had been exceeded. At 46% relative humidity, cotton fiber meter moisture contents average 7.0 ± 0.07% or about 0.3% higher than the corresponding average oven moistures for the same samples. The average meter fiber moisture reading was 9.3 ± 0.05% or about 0.8% higher than average oven fiber moisture content at the 70% relative humidity. Thus, in each case meter moisture readings were higher than oven moistures. The relative differences were, as observed during the calibration procedure, increased as fiber moisture increased.

**Fiber Stickiness**

Thermodetector tests for stickiness potential were determined on treated and untreated cotton at each fiber moisture content. Measurements were determined after the samples were conditioned for at least two days of at room temperature at 55 to 65% relative humidity. All of these cottons were rated in the non-sticky to slightly sticky ranges (Table 3). Ratings were based on the normal manual thermodetector stickiness scale of 0 to 4 sticky spots - non-sticky, 5 to 14 spots - slightly sticky, 16-24 spots - moderately sticky, and above 24 spots - extremely sticky. Considering the low levels of entomological sugars found on these lint samples, stickiness ratings were as expected. Stickiness results were mixed, within determination counting error, and averages were not significantly different between type, sugar concentration, or fiber moisture content according to a Duncan's Multiple Range test.

**NIR Spectroscopic Analysis**

Figure 1 shows a typical NIR absorbance spectrum of a cotton sample laden with entomological sugar along with its first and second derivative spectra at four different moisture contents. The absorbance plot shows that there are significant absorbance differences between the samples at different moisture contents. The most pronounced difference was between 1900 nm and 2000 nm, where water absorption band exists. Similar absorbance plots of cotton samples laden with different amounts of entomological sugars, however, showed very little discernable differences between sugar levels. The quantitative differences between different sugar levels as determined by HPLC were only a fraction of the differences between different moisture levels. It was concluded that the effect of moisture content on NIR spectrum is greater than the effect of sugar levels. This indicated that the prior knowledge of fiber moisture content is imperative in determining entomological sugar contents from NIR absorbance spectrum.

The first step in developing an empirical multilinear expression to predict the entomological sugar contents from NIR absorbance spectrum is to select a subset of significant wavelengths from 700 available wavelengths between 1100 and 2500 nm. One possible method to select these significant wavelengths is to observe the second derivative of the absorbance plot and select the wavelengths where pronounced negative peaks occur. Although the peaks in the absorbance plot are sometimes dull and broad, the second derivative plot shows sharp negative peaks where positive peaks occur in the absorbance plot. Preliminary tests showed that the second derivative plots of different amounts of entomological sugars at different moisture contents all show similar shapes, and their negative peaks occur at almost the same wavelengths within 2 nm of each other. Twelve wavelengths where largest negative peaks occur were selected as initial candidates for multiple regression analysis. These wavelengths, in order of magnitude, were at 2274, 1922, 2480, 2338, 2316, 2394, 2358, 2108, 1430, 1710, 1592, and 1482 nm.

The twelve wavelengths chosen and the moisture content of the sample were used as independent variables in multiple regression analysis to predict the total entomological sugar contents. The SAS procedure RSQUARE was used to examine a large number of models with varying numbers of independent variables. Several models with large $R^2$ values were selected to generate multiple regression equations using a REG procedure. Unfortunately, the $R^2$ value of the best regression equation was only 0.3492 when a single equation was applied to all tested samples. However, when the samples were divided into four ranges of moisture content, and a multiple regression equation is derived for each of the moisture content range, $R^2$ values became significantly higher. Figure 2 and 3 show the measured and predicted entomological sugar contents by a regression equation for moisture ranges between 4% and 5%, and between 9% and 10%, respectively. The regression equations employed all twelve wavelengths and the moisture content as independent variables. It was concluded that a reasonable multilinear relationship between NIR spectrum and the entomological sugar contents can be developed within a range of moisture content, if the fiber moisture content is known or can be measured.

The next step was to determine an optimum regression equation with a minimum number of wavelengths as independent variables. Table 4 shows the $R^2$ values of the best regression equation with varying number of independent variables for four moisture content ranges tested. Initial observation showed that the $R^2$ values did not increase significantly beyond seven independent variables. However, closer observation showed that different sets of wavelengths contributed at different moisture ranges, and all twelve wavelengths and the moisture content were needed to select four regression equations, one for each moisture range, even though each equation employs only seven variables. Considering that there is no reason to limit the number of variables when all twelve wavelengths have to be measured anyway, the thirteen variable models were determined as optimum multiple regression equations in this study.

Individual sugar contents of trehalulose and melezitose can also be predicted from the NIR spectrum and the moisture content. Using the same twelve wavelengths and the moisture content as independent variables, the $R^2$ values for trehalulose ranged from 0.5557 to 0.7829 for different moisture content ranges. The $R^2$ values for melezitose prediction equation ranged from 0.4427 to 0.8038 for different moisture content ranges. It was concluded that individual or total entomological sugar content can be estimated using the multiple regression equations with reasonable accuracy.

Instead of calculating exact sugar concentration, cotton samples can be classified into one of several levels of entomological sugar contents using a discriminant analysis. In this study, the entomological sugar contents ranged from zero to slightly under 0.2%. Although the sugar contents were generally lower than those observed in the field, a discriminant analysis procedure was developed using the acquired data. The same twelve wavelengths and the moisture content used in the regression analysis were used in the SAS DISCRIM procedure to compute a discriminant model. The entomological sugar levels were classified into two classes – "high" for more than or equal to 0.1% and "low" for less than 0.1%. The discriminant model was determined by a measure of generalized squared distance (Mahalanobis distance), based on within-group covariance matrices.

Table 5 shows the number of observations classified into two sugar levels using the discriminant model. Among the 120 observations, 107 samples were correctly classified for the success ratio of 89.2%. It should be noted that the result was obtained by a single discriminant model for all moisture content ranges. When four different discriminant models were developed and applied, one for each moisture range, all 120 samples were correctly classified for 100% success ratio.

The same procedure was applied to classify the levels of trehalulose and melezitose individually. The individual sugar levels were again classified into two classes – "high" for more than or equal to 0.1% and "low" for less than 0.1%. Tables 6 and 7 show the classification results. For trehalulose, 103 samples out of 120 were correctly classified for 85.8% success ratio. For melezitose, 108 samples were correctly identified for 90% success ratio. It was observed in Tables 5 through 7 that most samples with "high" sugar levels were classified correctly, and that most of the classification errors were in the "low" sugar range. This may indicate that

the discriminant analysis would perform well with higher sugar levels than investigated in this study.

## Summary and Conclusions

Near Infrared (NIR) spectroscopic analysis was used to study and develop theoretical relationships between different concentrations of entomological sugars on cottons. These entomological sugars on cotton were selected as combinations of trehalulose and melezitose. Batches of non-insect contaminated cotton samples were prepared with these two honeydew sugars separately and in 60/40 combination, at three different concentrations. Treated cottons conditioned at four different relative humidities were characterized by routine reducing sugar tests, thermodetector stickiness (TD) tests, NIR spectrophotometer analysis, moisture determinations by routine oven tests, portable moisture meter measurements, and high performance liquid chromatography (HPLC) for total and individual sugar contents. Overall measured levels of trehalulose, melezitose and mixtures of the two on the surface of treated cottons were much lower than targeted amounts. Low fiber moisture content, at time of blending, could have contributed to this problem. Samples at each conditioning moisture were also selected, sealed, and shipped to Stoneville, MS, for resistance moisture measurements.

NIR analysis showed that the effect of moisture content on NIR spectrum is greater than the effect of sugar levels, which indicated that the prior knowledge of fiber moisture content is necessary in determining entomological sugar contents from NIR absorbance spectrum. Twelve wavelengths, chosen from a second derivative of the absorbance plot, and the fiber moisture content were used as independent variables in multiple regression analysis to predict the total entomological sugar contents. Poor predictability was obtained when a single equation was applied to all tested moisture ranges. However, when the samples were divided into four ranges of moisture content, and a multiple regression equation is derived for each of the moisture content range, predictability increased significantly.

A discriminant analysis procedure was developed to classify cotton samples into two classes of the entomological sugar contents using the same twelve wavelengths and the moisture content used in the regression analysis. Among the 120 observations, 107 samples were correctly classified for the success ratio of 89.2% using a single discriminant model for all moisture content ranges. When four different discriminant models were developed and applied, one for each moisture range, all 120 samples were correctly classified for 100% success ratio. The discriminant analysis results also indicated that it would perform well with higher sugar levels than investigated in this study. This preliminary NIR analysis work may serve as a basic model to further refinement and eventual development of a rapid on-line screening test device to detect the presence and level of insect honeydew on cotton lint.

Lint moisture measurements taken on the portable meter in an operating range of 5 to 7% moisture were quite reliable. However, above 7%, meter moisture readings drifted higher as corresponding standard oven moisture measurements increased.

Results on resistance moisture meter measurement from the Stoneville, MS laboratory are pending.

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Table 1. Chemical analysis of a non-honeydew cotton treated with entomological sugars.

| | Perkins Test | | | HPLC Analysis | | | |
|---|---|---|---|---|---|---|---|
| Treatment | R. S. (%) | S. E. (%) | Treh. (%) | Treh. (%) | S. E. (%) | Mele. (%) | S. E. (%) |
| **Untreated** | 0.24 | 0 | 0 | 0 | - | 0 | - |
| **Trehalulose** | | | | | | | |
| Low | 0.34 | 0.023 | 0.10 | 0.08 | 0.004 | 0 | - |
| Medium | 0.40 | 0.035 | 0.16 | 0.14 | 0.006 | 0 | - |
| High | 0.46 | 0.045 | 0.22 | 0.17 | 0.031 | 0 | - |
| **Melezitose** | | | | | | | |
| Low | 0.24 | 0.012 | 0 | 0 | -0.08 | 0.011 | |
| Medium | 0.23 | 0.017 | 0 | 0 | -0.15 | 0.010 | |
| High | 0.22 | 0.009 | 0 | 0 | -0.18 | 0.010 | |
| **60/40 Mix** | | | | | | | |
| Low | 0.27 | 0.006 | 0.03 | 0.05 | 0.004 | 0.04 | .022 |
| Medium | 0.34 | 0.012 | 0.10 | 0.10 | 0.014 | 0.07 | .038 |
| High | 0.36 | 0.021 | 0.12 | 0.12 | 0.003 | 0.08 | 0.003 |

R.S. (%) = Standard reducing sugar test results
S.E. (%) = Calculated standard error of determination

Table 2. Average moisture contents of a non-honeydew cotton treated with entomological sugars at different relative humidity.

| | Portable Moisture Meter Reading | | | | | |
|---|---|---|---|---|---|---|
| | Oven Moisture | | | | | |
| Treatment | 24% RH* | 46% RH* | 70% RH* | 81% RH* | 46% RH* | 70% RH* |
| **Untreated** | 4.45 | 6.79 | 8.49 | 9.47 | 7.0 | 9.3 |
| **Trehalulose** | | | | | | |
| Low | 4.46 | 6.69 | 8.45 | 9.38 | 7.0 | 9.3 |
| Medium | 4.60 | 6.69 | 8.53 | 9.39 | 6.9 | 9.4 |
| High | 4.51 | 6.63 | 8.51 | 9.15 | 7.0 | 9.4 |
| **Melezitose** | | | | | | |
| Low | 4.67 | 6.80 | 8.51 | 9.32 | 6.9 | 9.4 |
| Medium | 4.62 | 6.67 | 8.58 | 9.44 | 7.0 | 9.4 |
| High | 4.52 | 6.68 | 8.47 | 9.41 | 7.1 | 9.3 |
| **60/40 Mix** | | | | | | |
| Low | 4.62 | 6.70 | 8.41 | 9.17 | 6.9 | 9.3 |
| Medium | 4.51 | 6.58 | 8.47 | 9.33 | 6.9 | 9.3 |
| High | 4.61 | 6.61 | 8.37 | 9.24 | 7.0 | 9.3 |

* No significant differences between sample by Duncan's Multiple Range Test.

Table 3. Thermodetector stickiness of a non-honeydew cotton treated with entomological sugars at different relative humidity.

| | Relative Humidity | | | | |
|---|---|---|---|---|---|
| Treatment | 24%$^{T}$ | 46%$^{T}$ | 46%$^{T}$ | 81%$^{*}$ | Average |
| **Untreated** | $0.3^{a}$ | $0.7^{a}$ | $1.3^{a}$ | 1.7 | 1.0 |
| **Trehalulose** | | | | | |
| Low | $2.0^{ab}$ | $3.3^{ab}$ | $3.0^{ab}$ | 5.3 | 3.4 |
| Medium | $4.0^{ab}$ | $4.4^{ab}$ | $5.7^{c}$ | 5.0 | 4.8 |
| High | $4.3^{ab}$ | $5.0^{ab}$ | $4.0^{ab}$ | 3.3 | 4.9 |
| **Melezitose** | | | | | |
| Low | $5.0^{ab}$ | $4.7^{ab}$ | $3.7^{ab}$ | 4.7 | 4.5 |
| Medium | $5.3^{c}$ | $5.0^{ab}$ | $6.0^{c}$ | 6.7 | 5.8 |
| High | $5.7^{c}$ | $3.3^{ab}$ | $4.3^{ab}$ | 6.3 | 4.9 |
| **60/40 Mix** | | | | | |
| Low | $4.3^{ab}$ | $4.0^{ab}$ | $2.7^{ab}$ | 5.0 | 4.0 |
| Medium | $4.0^{ab}$ | $5.3^{ab}$ | $5.3^{ab}$ | 4.0 | 4.7 |
| High | $3.7^{ab}$ | $5.0^{ab}$ | $6.7^{c}$ | 2.3 | 4.4 |

* No significant differences between sample by Duncan's Multiple Range Test at the 95% confidence level.
T - Duncan's Multiple Range Test means followed by same letter are not signficantly different at the 95% confidence level

Table 4. $R^2$ values of the best regression equation with varying numbers of independent variables for four moisture content ranges tested.

| Number of Variables | Range of Moisture Content 4%-5% | 6%-7% | 8%-9% | 9%-10% |
|---|---|---|---|---|
| 3 | 0.3738 | 0.3655 | 0.1932 | 0.3785 |
| 5 | 0.5419 | 0.5023 | 0.4414 | 0.5893 |
| 7 | 0.6039 | 0.6145 | 0.4915 | 0.7181 |
| 9 | 0.6176 | 0.6413 | 0.5060 | 0.7318 |
| 11 | 0.6386 | 06461 | 0.5125 | 0.7415 |
| 13 | 0.6437 | 0.6471 | 0.5150 | 0.7422 |

Table 5. Discriminant classification result for total entomological sugar contents.

| | Number of | Classified into | |
|---|---|---|---|
| Sugar Level | Samples | High | Low |
| High | 76 | 74 | 2 |
| Low | 44 | 11 | 33 |
| Total | 120 | 85 | 35 |

Table 6. Discriminant classification result for trehalulose contents

| Sugar Level | Number of Samples | Classified into High | Low |
|---|---|---|---|
| High | 44 | 41 | 3 |
| Low | 76 | 14 | 62 |
| Total | 120 | 55 | 65 |

Table 7. Discriminant classification result for melezitose contents

| Sugar Level | Number of Samples | Classified into High | Low |
|---|---|---|---|
| High | 28 | 28 | 0 |
| Low | 92 | 12 | 80 |
| Total | 120 | 40 | 30 |

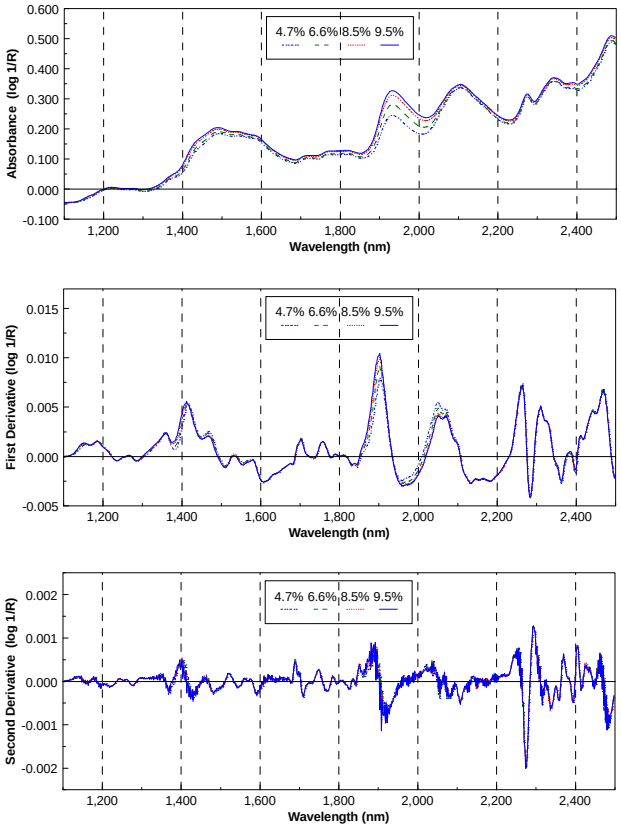


Figure 1. Spectral aborbance and its first and second derivative spectra of cotton laden with 0.12% trehalulose and 0.08% melezitose at four different moisture contents.

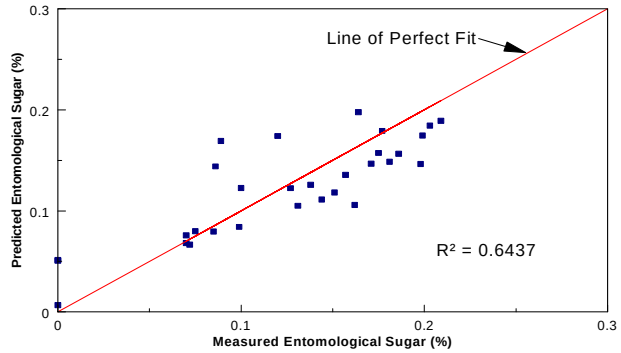


Figure 2. Measured and predicted entomological sugar contents when moisture contents range between 4% and 5%.

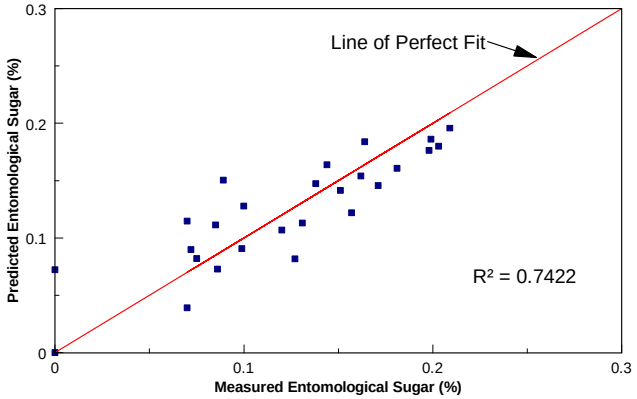


Figure 3. Measured and predicted enomological sugar contents when moisture contents range between 9% and 10%.