

## TEXTILE TECHNOLOGY

### Upland Cotton Surface Amino Acid and Carbohydrate Contents vs. Color Measurements

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#### ABSTRACT

**Upland cotton is naturally white, with its yellowness (+b) rating highly influencing its economic value. Field conditions, microorganisms, and growth problems can cause cotton to become discolored at harvest, which has historically been thought to indicate a decrease in product quality. Previous research has suggested that some reactions between amino acids and carbohydrates on the surface of cotton fibers may lead to color development after harvest during certain storage and shipping conditions. There has been a lack of research evidence to understand how initial amounts of those surface constituents present at harvest may indicate the propensity for potential future changes in +b ratings. Due to the monetary implications, it is important for those in the cotton industry to better understand exactly how detrimental the +b value is on the functionality of the cotton. This study aimed to identify potential relationships between the post-harvest surface amino acids and carbohydrates content with color rating values to gain insight using High Volume Instrument (HVI), a portable spectrophotometer, ion chromatography, and a ninhydrin test to compare amino acid and carbohydrate content of 45 upland cotton samples with their color measurements: +b, Rd, and L\*a\*b\*. A correlational statistical analysis found a quadratic relationship between amino acid content and +b; and highly positive correlations between amino acids and +b ratings: 0.8607; and b\* values: 0.820 (p<0.05).**

Cotton color has a large influence on the economic value of the commodity. Recently, stakeholders have raised concerns about the monetary losses due to cotton color development during the time from

harvest to its arrival at certain processing locations. Studies on cotton fiber properties, which influence cost, have shown that yellowness, or the +b rating, can undergo significant changes during storage and shipping (Gamble, 2007). Upland cotton is naturally white; however field weathering and microorganisms can cause cotton to discolor. If cotton growth is stunted, the fibers can also experience increased yellowness. Unfortunately, color deviation from the bright white character of cotton has historically been thought to indicate an automatic decrease in product quality, whether or not the color development happened during plant growth or during storage/shipping (Wakelyn et al., 2007).

With respect to field-to-fabric applications, certain aspects of cotton color may be a key contributor to the ultimate downstream efficiencies, having been shown to have substantial impact on the dyeability of fabrics; and bleaching methods do not fully compensate for dye differences (Gamble, 2007). The problem of barré, a color shade variation in dyed fabrics resulting in defect stripes, has recently been found to be influenced by the color strength of the cotton used in the dyed samples (Ashraf et al., 2014). In that study, the color values of the dyed samples correlated positively with +b values, and correlated negatively with the brightness values (Rd) of the raw cottons used to make the samples (Ashraf et al., 2014).

There have been relatively few multivariate studies done to account for the interrelationships of a large number of cotton fiber properties at once, but most have focused on properties obtained from the High Volume Instrument (HVI) and Advanced Fiber Information System (AFIS), as these instruments have been the cornerstone of modern cotton testing and grading (Ghosh et al., 2015). Ghosh, et al. (2015), found that +b correlates positively with micronaire and short fiber index and correlates negatively with Rd, length uniformity, upper-half mean length, fiber elongation and fiber strength. Despite correlations of +b with many important cotton properties, it is yet to be determined if cotton color development is a definitive indicator of low quality cotton. In fact, other studies have posited that color development over time was likely due to surface microbial activ-

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ity and had no real bearing on yarn quality (Gamble, 2007). The question then remains if cotton color development is the result of surface-related chemical reactions or if it is a result of the inherent physical properties that effect processing.

Studies have postulated that reactions between amino acids and carbohydrates on the surface of the cotton fibers may lead to color development through a reaction similar to the Maillard reaction, which results from chemical condensation between a reducing sugar and an amino acid (Gamble, 2008). The initial amounts of amino acids and carbohydrates on the fiber surface after harvest may thereby help explain if and how cotton color relates to cotton structure properties. Carbohydrate levels have, on occasion, been shown to correlate with an increase in +b over time, and a previous study on increases in +b in relation to storage conditions indicated that some carbohydrate levels did change due to mimicked storage conditions and heating; but the study did not measure surface amino acids (Ellsworth et al., 1999; Gamble, 2008; Rathert, 1983).

Indications that surface chemicals may be linked to color development might be found in cotton's physical formation. In general, cotton fiber forms in four stages: initiation, elongation, secondary cell wall thickening and maturation, and it has been found that proline, glycine and tyrosine-rich proteins have been identified as major players in cell wall structure, from the inside out (Maliyakal and Keller, 1995). Previously 18 amino acids were found in the upland cotton plant (*Gossypium hirsutum*); specifically the boll contains: aspartic acid, asparagine, threonine, serine, glutamine, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, and arginine. The majority of free amino acids detected were glutamic acid, aspartic acid, valine, serine and threonine (Burks and Earle, 1965; Wakelyn et al., 2007). Inositol, glucose, fructose and sucrose have been found to be the major carbohydrates found naturally in and on cotton; while trehalose, maltose, trehalulose and melezitose are usually present due to pests and fungi (Hequet and Abidi, 2006). If color development is solely a surface issue, these constituents should be found on the cotton surface and have some relation to color ratings. Moreover, if the interactions of these chemicals do not majorly affect cotton's physical processing efficiency, it would be beneficial to those invested in the cotton industry to have that

information. The time after harvest is when cotton is initially judged for its value; therefore, a better understanding of the initial surface relationships between non-cellulosic surface chemicals is needed.

In the study by Burks and Earle mentioned above, asparagine, glutamine and proline (along with ammonia) were found to comprise roughly 70% of the total ninhydrin-positive components identified within the cotton boll, or square (Burks and Earle, 1965). Again, it was postulated that high surface amino acid and carbohydrate content should yield high +b ratings, because that would indicate the initial, post-harvest availability of the chemicals that induce color change reactions, such as the Maillard reaction.

This work measured the surface amino acid and carbohydrate content of 45 upland cotton samples for comparison with color measurements +b, Rd, L\*, a\*, and b\*, obtained from HVI and a portable spectrophotometer, in order to investigate correlations between fiber surface non-cellulosic materials and cotton fiber color. While spectrophotometers do not measure and report the cotton rating components of Rd and +b directly, most measure and report the more universally accepted, three-dimensional color spaces, such as L\*a\*b\* (or CIELAB) (Rodgers et al., 2013). L\* is a measurement of lightness (or greyness), a\* is the measurement of greenness to redness, and b\* is the measurement for blueness to yellowness (Rodgers et al., 2013). A more universally recognized cotton color measurement standard is desirable, so the cotton surface amino acid and carbohydrate levels should be compared to the L\*a\*b\* values as well; especially since resultant dyed goods are measured in L\*a\*b\* (Aspland and Williams, 1991; Thibodeaux et al., 2008; Xu et al., 1998). Finding correlations between these conventional spectrophotometer measurements and cotton surface chemicals is also of use because the technological advances in handheld spectrophotometers (which include measurements of the full visible spectrum) have made it easy to assess cotton color cheaply and easily outside of the laboratory (warehouse, field, etc.).

Since color ratings are typically accomplished shortly after harvest, this study aimed to assess the impact of amino acids on color values at that time. Because color is such a huge impact on cotton's monetary value, it is highly marketable to the cotton industry to understand if cotton yellowness is not necessarily always detrimental to fiber processing.

Cotton's yellowness, in some cases, may simply be a result of the chemical building blocks of the fibers interacting otherwise inconsequentially; or even from the interaction with trash content. Upland cotton now provides over 90% of the current world production of raw cotton fiber, so only upland cotton was considered for this study (Wakelyn et al., 2007). A ninhydrin test for amino acids and an ion chromatography analysis for carbohydrates utilized at the same time as the color measurements were obtained, relatively shortly after harvest, allowed for the cotton's surface chemical content versus color assessment; before any long term storage and/or long term shipping actions could potentially effect color development (Rodgers et al., 2013). A statistical analysis was used to analyze information about the different color and chemical parameters inherent to the cotton fibers that would otherwise be difficult to compare (Kazama et al., 2015).

## MATERIALS AND METHODS

**Materials.** Inositol (98+) was obtained from Acros Organics (New Jersey, USA). Trehalose, D-(+)-trehalose dehydrate; glucose, D-(+)-glucose, fructose (D-(-)-fructose; sucrose, melezitose, ( $\alpha$ -D-glucopyranosyl-[1 $\rightarrow$ 3]- $\beta$ -D-fructofuranosyl-[2 $\rightarrow$ 1]- $\alpha$ -D-glucopyranoside) hydrate, (99+); raffinose, D-(+)-raffinose pentahydrate; maltose monohydrate and acetic acid were purchased from Sigma-Aldrich Corporation (St. Louis, MO). Trehalulose, (90%) 1-O- $\alpha$ -D-glucopyranosyl-D-fructose was purchased from Chem Service Inc. (West Chester, PA). Glycine and ninhydrin (2% solution) were purchased from Sigma-Aldrich Corporation (St. Louis, MO). Sodium hydroxide (50% solution) and sodium acetate anhydrous were obtained from Fischer Scientific (Fair Lawn, NJ). Acrodisc syringe filters with 13mm nylon membranes (diameter 13 mm, pore size 0.2  $\mu$ m) were obtained from Thermo Scientific (Rockwood, TN). The Corning LS Vortex Mixer was purchased from Corning Incorporated (Corning, NY), and the water was distilled, deionized, and then further purified with a Millipore Direct-Q 3UV, 8 system from EMD Millipore Corporation (Billerica, MA).

**Cotton Samples.** Forty-five raw upland cotton samples exhibiting a range of +b values were obtained from the Agricultural Marketing Service (AMS) Cotton and Tobacco Program in Memphis, TN. All samples were measured in triplicate for

amino acids and spectrophotometer  $L^*a^*b^*$  values and five times for Rd and +b values from HVI. The samples were randomly selected from the 2016-17 U.S. cotton crop and originated from Texas, Mid-South and Southeast growing regions. Each bulk sample was collected from commercially ginned 500-pound cotton bales. All bale samples were from either picker or stripper harvested fields and saw-ginned with no further modifications. It is unknown which of the harvesting methods was used on each bale/sample. These were all commercially grown cottons that presumably would have been harvested at full maturity and given to the AMS facility within 48 hours of harvesting. The samples were specifically chosen to give a range of +b values, regardless of their growing location in order to assess only resultant fiber color with regards to their carbohydrate and amino acid contents.

**Cotton Sample Preparation.** The samples were kept in a refrigerated storage area at 4°C to slow chemical surface interactions until all testing methods were completed. For analysis, each cotton sample aliquot was treated with respect to the corresponding analytical and/or physical testing method being employed.

**High Volume Instrument (HVI).** The yellowness (+b) and brightness (Rd) values were measured (in quintuplicate) using loose cotton on the SRRC Uster HVI-1000 (Uster Technologies Inc., Knoxville, TN), according to standard test methods (ASTM, 2005). HVI color references were obtained from AMS. The HVI values +b and Rd are measured optically by color filters and used in the HVI® Color Chart for cotton grading: +b is a yellowness scale from 4 to 18 and Rd is a measure of grayness (how light or dark a sample is) on a scale of 40 to 90.

**Spectrophotometer.** The  $L^*a^*b^*$  color evaluation of the upland cottons was accomplished using the HunterLab MiniScan EZ (MSEZ) (Hunter Associates Laboratories, Inc., Reston, VA) portable spectrophotometer in with a D65/10° daylight simulator illuminant and observer. Samples were folded for sufficient opacity before each measurement.  $L^*$ ,  $a^*$  and  $b^*$  values are derived from the CIELAB color space and represent the lightness, greenness-to-redness, and blueness-to-yellowness, respectively.  $L^*$ ,  $a^*$  and  $b^*$  are derived mathematically from their relationships in a three dimensional real number space. Measurements were taken on five different spots of a cotton tuft, and results were averaged for a given sample.

**Carbohydrate Analysis using Ion Chromatography.** For carbohydrate analysis by ion chromatography, one gram of cotton was comprised by pinching small portions from the total sample in order to increase efficient sampling (Peralta et al., 2016). The raw cotton sample was placed in a 50 mL centrifuge tube and 20 mL of ultrapure deionized water was added. The tube was capped and vortexed at ~ 5,000 RPM for 30 seconds, then again at ~3,500 RPM for five minutes. The water extract from the vortexed samples was then filtered through a 0.2  $\mu\text{m}$  syringe filter to remove any cotton fibers and particulate and transferred to a 1.5 mL auto-sampler glass vial for analysis by ion chromatography. Some filtered extract was saved for immediate use in a ninhydrin amino acid test (Section 2.7).

Ion chromatography was performed on a Dionex DX-5000 instrument (Thermo Scientific, (Rockwood, TN) using pulsed amperometric detection, and elution was carried out using a flow rate of 0.80 mL/min through a Dionex CarboPac PA-1 (4  $\times$  250 mm) column. An isocratic eluent was employed using 200 mM NaOH for 15 minutes. The column and compartment temperatures were set at 30°C. An aqueous stock standard of eight sugars (inositol, trehalose, glucose, fructose, sucrose, melezitose, raffinose and maltose) was created. The trehalulose standard was prepared and run on the ion chromatography instrument separately, since the trehalulose syrup was only 90% pure (which is common, by industrial standards) and contained levels of glucose, fructose and an unidentified component. The analytics software used was Dionex Chromeleon 7.2 CDS.

**Amino Acid Analysis.** Amino acids were measured using a ninhydrin test whereby a condensation reaction occurs to form Ruhemann's purple ( $\lambda_{\text{max}}$  570 nm;  $\epsilon = 22\ 000$ ) (diketohydrindylidene-diketohydrindamine, DYDA); measurable by UV-vis spectroscopy (Friedman, 2004). For the analysis, a stock solution of 50  $\mu\text{M}$  glycine solution in trace acetic acid and water was prepared in order to create ninhydrin reaction standards. First, a 0.01 M glycine solution was created by dissolving 0.0751g of glycine in 100 mL of nanopure water. Then, 1 mL of the 0.01M glycine solution was added to a 200 mL volumetric flask. One hundred microliters of glacial acetic acid was added, and the solution was diluted to 200 mL with nanopure water.

For the standards, the stock solution of 50  $\mu\text{M}$  glycine solution was used to create standards, each reacted with 1 mL of ninhydrin: Standard A contained 0.5 mL glycine stock, 1.5 mL water and 1 mL

of ninhydrin in a scintillation vial to yield a 3.125  $\mu\text{M}$  glycine in ninhydrin solution. Subsequent standards were created with varying amount of glycine stock and water along with the 1 mL of ninhydrin. A blank was created by using 2 mL of water and 1 mL of ninhydrin. The reaction took place by placing the scintillation vial on a hot plate to boil for 10 minutes. After boiling, the solution was allowed to cool and 5 mL of 95% ethanol was added.

For the sample preparation, the cotton water extract was obtained through the same vortex and filter process as described in Section 2.6. In this case, 2 mL of the cotton water extract was placed in the scintillation vial along with the 1 mL of ninhydrin solution, and the heating process was carried out. All scintillation vials with the reacted solutions were taken for UV-Vis analysis as described in Section 2.8.

**UV-Vis Spectroscopy.** The UV-Vis absorbance spectra were acquired using a Cary 50 spectrophotometer instrument (Varian, Palo Alto, CA) with a scan spectral program. Three replicates were taken for the ninhydrin reacted cotton water extracts over the spectral range of 200 to 800 nm, in 5 nm increments, using a probe attachment.

**Data Analysis.** Relationships between the color values, the amino acids, and the carbohydrates were determined using the 45 upland cotton samples. Correlation analysis, regression analysis and cluster analysis were used to describe these relationships. All analyses were performed using JMP 13 (SAS Institute Inc., Cary, NC).

## RESULTS AND DISCUSSION

**Correlational Analysis – Amino Acids versus Carbohydrates.** To better understand whether or not a color change (potentially Maillard-type) reaction is occurring on the surface of cotton fibers causing color development, it is helpful to know the extent of the amino acids and carbohydrates present on the fibers at the time of initial color grading. To detect these amino acids in a cotton water extract, a simple ninhydrin test was employed to detect free amino acids. It was a supposition that cotton with larger +b values would show initial increased amino acid and carbohydrate content in order to fit with the previous ideas that those chemicals are needed for the reactions which are conducive to further color change during storage and shipping (Gamble, 2007). It is important to note however, that the ninhydrin test does not test for the presence of proline, as it reacts

differently with ninhydrin. Tyrosine, phenylalanine, tryptophan and lysine give reduced photometric results compared to the equivalent standard as well, but the results are nevertheless a good estimation of their existence in the water extract (Yemm et al., 1955).

Across the 45 samples, amino acids were found to be present in a range of 1.68 to 34.69  $\mu\text{mol}$  per one gm of cotton, with a mean of  $10.09 \pm 7.96 \mu\text{mol}$ . The carbohydrates varied greatly in range and in mg per one gm of cotton, with inositol being the only sugar present possessing a Gaussian distribution. Table 1 shows the correlations among the  $\mu\text{mol}$  of amino acids and the mg of carbohydrates present in the water extracts (of one gram of cotton) for the 45 samples. Of the eight sugars tested using ion chromatography, raffinose was not detected in significant levels, so it was not part of the analysis. Correlations greater than 0.29 were significant ( $p \leq 0.05$ ). Very large, or significant, correlations (greater than 0.500 positive or negative) are in bold type. Some of the correlational findings when relating carbohydrates present on cotton fibers to one another has already been previously reported; such as the highest positive correlation of 0.950 being between trehalulose and melezitose ( $p < 0.05$ ). This correlation was in line with previous results, since trace amounts of melezitose have been found when certain levels of trehalulose were also present on cotton fibers; due to both sugars being components of insect honeydew (Hequet and Abidi, 2006; Peralta et al., 2016). Also, to be expected, the plant sugars inositol, glucose, fructose and sucrose all show high levels of positive correlation with one another, as they are all major plant carbohydrates.

All of the sugars, with the exception of inositol, show negative and negligible correlations with the amino acids. Inositol also showed the highest, though still relatively insignificant, correlation with the

amino acids. ( $p < 0.05$ ) These findings indicate that if a surface reaction is indeed happening to influence the initial cotton color, it is not immediately observable by an amino acid:carbohydrate correlation. Unless substantial correlations are found between surface amino acids vs. color parameters and/or surface carbohydrates vs. color parameters, post-harvest cotton color may be more dependent on the internal physical formational characteristics and less dependent on surface interactions.

**Correlational Analysis – Carbohydrates versus Color Measurements.** After finding low correlational evidence between amino acid and carbohydrate content extracted from cotton fibers, relationships between the carbohydrates and the color measurements from the HVI and spectrophotometer: +b, Rd, L\*, a\* and b\* were investigated. Again, in Table 2, substantial high correlations ( $\leq -0.500$  and  $\geq 0.500$ ) between measurements of initial cotton color and carbohydrates are in bold type.

The relationships between the HVI (+b and Rd) and spectrophotometer (L\*, a\* and b\*) obtained color measurements have also been previously researched and mathematically determined, but it is still interesting to take note of the high positive and negative correlations between the measurement types given here, especially correlations with +b, which may be influential in cotton’s economic value (Thibodeaux et al., 2008). The +b range for the 45 sample cottons was 6.9 to 12.8 with a mean of  $9.29 \pm 1.54$ . The mathematically converted values of L\* and b\* taken from Rd and +b values are usually higher than the spectrophotometrically obtained L\* and b\* values; the difference has been explained by instrumental glass covering, instrument calibration differences and incident light sources which are yellow (Aspland and Williams, 1991).

**Table 1: Correlation values between the eight carbohydrates and total amino acid content present in the water extracts**

Amino Acid (AA)	<b>1.000</b>								
INOSITOL (I)	0.250	<b>1.000</b>							
TREHALULOSE (T)	-0.009	-0.315	<b>1.000</b>						
GLUCOSE (G)	-0.132	<b>0.584</b>	-0.106	<b>1.000</b>					
FRUCTOSE (F)	-0.114	<b>0.629</b>	-0.146	<b>0.760</b>	<b>1.000</b>				
TREHALULOSE (TRE)	-0.029	0.348	0.018	0.053	0.458	<b>1.000</b>			
SUCROSE (S)	-0.135	<b>0.549</b>	-0.156	<b>0.791</b>	<b>0.726</b>	-0.004	<b>1.000</b>		
MELEZITOSE (MEL)	-0.016	0.227	0.041	-0.118	0.312	<b>0.950</b>	<b>-0.155</b>	<b>1.000</b>	
MALTOSE (M)	-0.057	0.325	0.302	0.375	0.328	-0.036	<b>0.598</b>	-0.098	<b>1.000</b>
	AA	I	T	G	F	TRE	S	MEL	M

**Table 2: Correlation values between the eight carbohydrates and color measurements**

	L*	a*	b*	Rd	+b
L*	<b>1.000</b>				
a*	<b>-0.839</b>	<b>1.000</b>			
b*	<b>-0.515</b>	<b>0.824</b>	<b>1.000</b>		
Rd	<b>0.996</b>	<b>-0.827</b>	-0.503	<b>1.000</b>	
+b	<b>-0.704</b>	<b>0.899</b>	<b>0.949</b>	<b>-0.701</b>	<b>1.000</b>
INOSITOL (I)	0.262	-0.088	0.302	0.271	0.164
TREHALOSE (T)	-0.440	0.293	-0.007	-0.428	0.104
GLUCOSE (G)	0.432	-0.230	-0.008	0.443	-0.134
FRUCTOSE (F)	0.393	-0.398	-0.126	0.388	-0.183
TREHALULOSE (TRE)	0.206	-0.342	-0.178	0.183	-0.157
SUCROSE (S)	0.410	-0.259	0.005	0.422	-0.139
MELEZITOSE (MEL)	0.138	-0.296	-0.189	0.118	-0.152
MALTOSE (M)	0.016	-0.009	0.113	0.027	0.038

The L\* measurement shows low to moderate positive correlations with all sugars except for trehalose. According to prior reports., fungi is the main supplier of trehalose on cotton and it appeared to play a large role in the increase of yellowness, +b, because the fungi use surface materials, such as sugars for growth; and while there was no substantial correlation between trehalose and +b, the negative correlation between brightness values (Rd: -0.440 and L\*: -0.428) and trehalose may be of note (Gamble, 2007; Hequet and Abidi, 2006). More importantly, in Gamble's study, a decrease in glucose was thought to be explained by fermentation of fungi on the fiber surface as they consume the carbohydrates, while no significant changes were observed for Rd but yellowness (+b) substantially increased (Gamble, 2007). Amongst the other sugars, there were only negligible correlations with all of the color measurement values; however, the trends were mostly negative relationships with a\*, b\* and +b and mostly positive relationships with Rd and L\* brightness measurements ( $p < 0.05$ ).

**Correlational Analysis – Amino Acids versus Color Measurements.** Cotton fibers are composed of an outer primary wall which is made up of proteins, pectins and waxes and contains ~55% cellulose, and an inner, more substantial secondary wall, which is almost entirely cellulose, but it is amino acids and carbohydrates that are largely responsible for the successful growth of the cotton plant, and while the initial cotton fiber color could be due to internal amino acids and carbohydrates located in the lumen,

outer surface amino acids could play the biggest role (Mitchell et al., 2005). A straight-forward method to detect most free amino acids present on the surface of the cotton fiber is a water extraction and subsequent ninhydrin test.

Ninhydrin is a chemical used to detect primary and secondary amines and when reacted with those amines, it produces a “Ruhemann's Purple” chromophore. Ninhydrin is commonly used to detect fingerprints by reacting with the terminal amines of lysine residues in peptides and proteins of sweat oils. The only drawback of using ninhydrin for amino acid detection on cotton fibers is that it does not react with proline, and proline is a prominent amino acid in cotton. Despite this, the ninhydrin is an efficient proof-of-concept test to determine trends or correlations between amino acids and cotton color. Table 3 shows the correlation found when comparing amino acid content (minus the proline) in the water extracts versus cotton color measurement values, with substantial high correlations ( $\leq -0.500$  and  $\geq 0.500$ ) in bold type.

**Table 3: Correlation values between the amino acid levels and color measurements**

Color Measurements	Amino Acids
+b	<b>0.861</b>
L*	<b>-0.593</b>
a*	<b>0.771</b>
b*	<b>0.820</b>
Rd	<b>-0.589</b>

As evident by the table, every correlation between amino acids and +b, Rd, L\*, a\* and b\* was significant (greater than  $\pm 0.500$ ). It is striking that the highest positive correlation was between amino acids and +b: 0.861 ( $p < 0.05$ ), followed closely by b\*: 0.820 ( $p < 0.05$ ). This indicates that there is some amino acid present on the surface of the cottons, not just in the lumen, that contributes to initial cotton color and color development. This finding proved our hypothesis to be true: that there should be an increase in amino acid content where there was an increase in the +b rating if color development is largely due to surface chemical reactions. Furthermore, for the cotton samples where there is an increased amount of amino acids present shortly after harvest, there is an increased chance for further color development, due to Maillard-type color change reactions, over time.

In a previous multivariate study that used a k-means analysis to compare harvesting methods (manual, picker and stripper) versus cotton characteristics, it was found that the manual picking method showed the highest means for fiber characteristics that were considered desirable, such as upper half mean length, length uniformity, tensile strength, fiber percentage and Rd; however, it also showed the highest mean for +b (Kazama et al., 2015). Interestingly, amino acids are notoriously easy to transfer, even being highly present in the oils present in human skin, so there may be some transfer of surface amino acids onto cotton fibers due to touch (Friedman, 2004).

Amino acids levels also had a significant negative correlation with Rd (-0.589) and L\* (-0.593), the brightness measurements Ashraf et al. (2014). concluded that yarns made from cotton with high Rd and lower +b values give lighter color depth after dyeing, and vice versa. Whether amino acids possess inversely proportional relationships with those measurements that facilitate color development is yet to be seen, but an investigation into amino acid content versus those dyeing affects would be prudent. Previous studies also deemed the a\* measurement as relatively unimportant with respect to cotton color measurements; however, this study found a strong positive correlation between a\* and +b (0.899) (Aspland and Williams, 1991; Thibodeaux et al., 2008). Moreover, the a\* value showed a highly positive correlation with the amino acid content (0.771,  $p < 0.05$ ).

Such a strong correlation of +b (and other color measurements) versus amino acid content lends to the idea that there are indeed non-microbial surface chemical reactions that are responsible for cotton color and any changes in cotton color are not solely microbial induced (Gamble, 2008). Since the Maillard reaction may occur at temperatures lower than a caramelization reaction (caramelization occurs at 110°C for fructose and 160°C for glucose (Clemens et al., 2016)), amino acids may be highly influential on initial +b ratings and driving factors of any further changes in +b over time (Gamble, 2008). Even though strong linear relationships have previously been assigned to decreases in glucose and fructose versus an increase in +b under simulated ageing and storage condition parameters; the amino acid content may very well be the predictive variables for how much those sugars can actually react (Gamble, 2008). Gamble found that glucose content as a function of heating time at 70°C gave a reaction rate constant that did not fit a simple exponential function, and the data fit a bi-exponential equation much better; indicating that either glucose reacted in two separate pathways or that the reaction was not first order (Gamble, 2008). The work cited a need to determine surface amino acid content.

**Prediction of Amino Acids using +b.** The relationship between amino acids and +b was further investigated and it was found that although there was a Gaussian distribution for +b values for the 45 samples, there was a skewed distribution for amino acid content with increasing +b (Figure 1). A Kolmogorov's D goodness-of-fit test at a significance level  $\alpha = 0.1$  confirms that the amino acid content follows a LogNormal distribution in Figure 2.

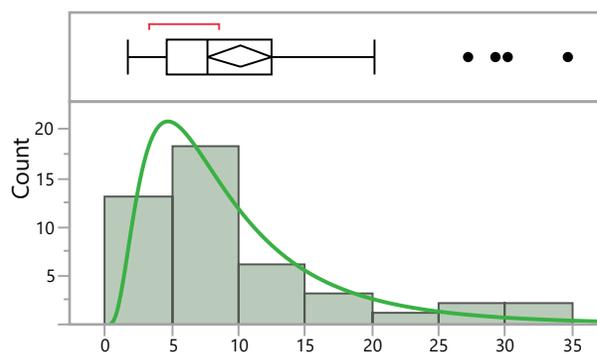
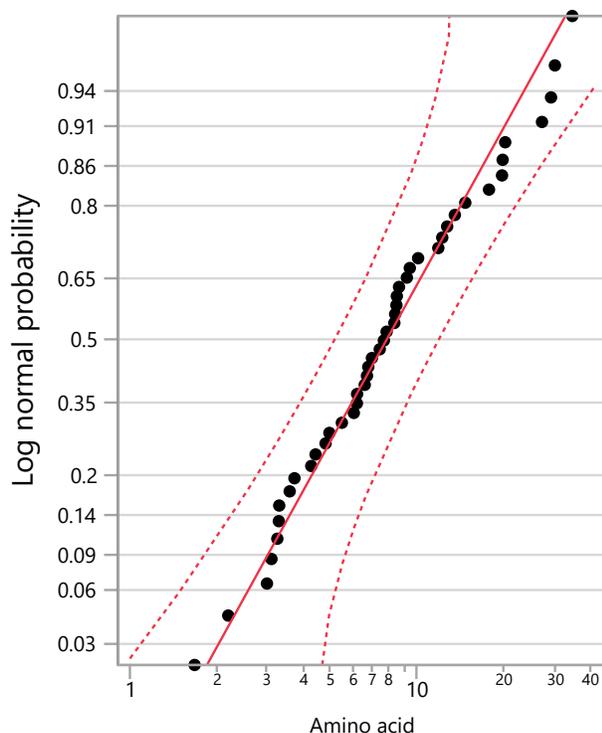
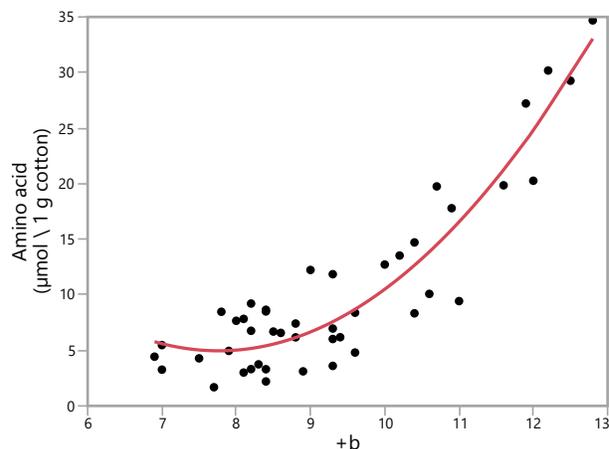


Figure 1: Histogram of amino acid ( $\mu\text{mol}$ ) content on 45 upland cotton samples.



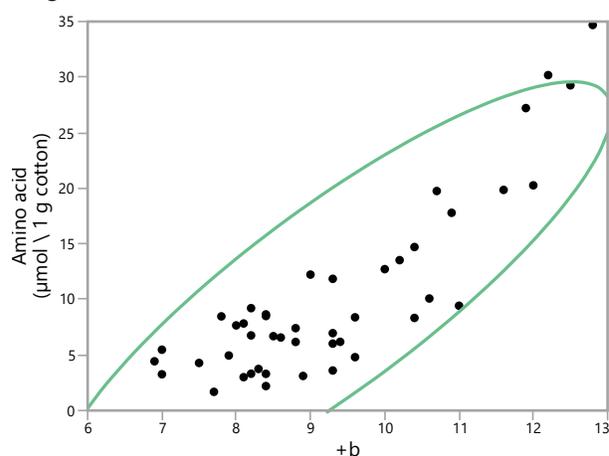
**Figure 2: Probability diagnostic plot of amino acid content.**

When a quadratic (polynomial, degree = 2) fit was chosen for the amino acids versus +b data,  $R^2$  equaled 0.8612; thus indicating a better fit than just a simple linear regression. Figure 3 shows the quadratic fit of amino acids vs. +b. Again, this is noteworthy because this relationship between amino acids and +b suggests that surface reactions are responsible for the color ratings of cotton and those reactions may not have any influence on the processing performance of the fibers. Any further color development over long-term storage and long term shipping conditions could further corroborate the findings that surface chemical reactions cause cotton yellowing; not a degradation of the physical fiber properties. Thus, cotton samples with high initial amounts of amino acids could prove the most vulnerable to color change, but not necessarily vulnerable to decreased value. Notwithstanding, it is unknown whether these surface reactions could be brought about by amino acid rich trash or foreign matter present on the surface of the cotton, especially those causing neps, such as seed coat, leaves or other plant matter. Cotton seeds have been shown to increase in amino acid content well into the flowering stage of the cotton plant development; and any seed fragments or motes deposited onto the fibers during harvesting and ginning could account for the increase in surface amino acids perpetuate color development (Elmore and Leffler, 1976).



**Figure 3: Quadratic fit of amino acid versus +b.**

Upon further bivariate diagnostics of amino acids versus +b, the normal ellipse contour plot ( $p=0.950$ ) indicated nearly all of the amino acid vs +b pairs followed bi-variate normal distribution since greater than 95% of the pair points lie within the 95% constant probability density contour ellipse (Figure 4).



**Figure 4: Bivariate normal ellipse of amino acid versus +b,  $p<0.05$ .**

**Clustering Upland Cotton Samples Based on Amino Acid, Carbohydrate and Color Values.** A principle component plot on correlations between amino acids, carbohydrates and cotton color measurements overall (Figure 5) further illustrates how Component 1 spans the most variation in the data; with the  $a^*$ ,  $b^*$ , and +b color measurement values and amino acid content clustering together (with amino acid fit directly in between +b and  $b^*$ ) on the left side, while all of the carbohydrates (except trehalose) and the brightness color values  $R_d$  and  $L^*$  clustered on the right side. Component 2, which spans the second most variation in the data, separated trehalose from all other values. Visually, it was evident that of the

non-cellulosic surface materials present on the cotton surface values (other than non-tested sugars, waxes, metals and pectins), the amino acids have the highest correlations with the color value that has the most influence on cotton color valuation, +b.

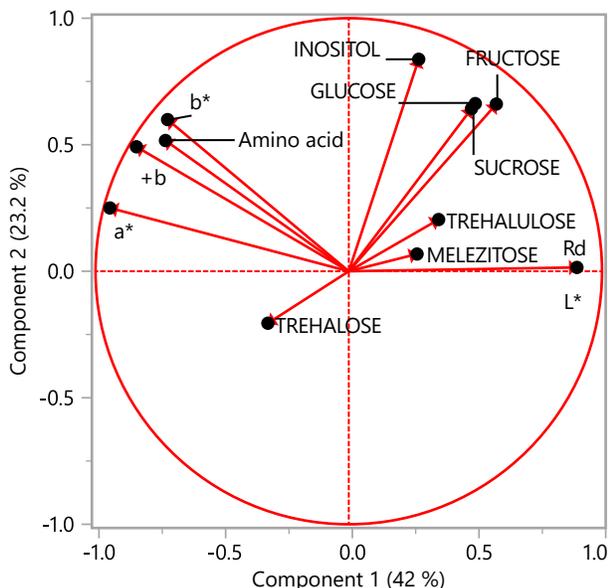


Figure 5: Principle component analysis on correlations of the cotton measurement data.

### CONCLUSIONS

By using correlational and bivariate analyses of color measurements, amino acid and carbohydrate content values present for 45 upland cotton samples, statistical correlations to better understand the relationships between those characteristics were determined. Amino acid content using the ninhydrin test was found to have a Log-Normal distribution, and when compared to color measurement values, it highly, positively correlated with +b values: 0.861; and b\* values: 0.820 ( $p < 0.05$ ). Amino acid content had a significant negative correlation with Rd and L\* values, -0.589 and -0.593, respectively. When using a bivariate linear fit of amino acids by +b, the  $R^2$  was 0.741. Amino acids had far better correlations with the color measurement values than all of the carbohydrates tested in this study. Therefore, it stands to reason that cotton samples with increased amino acid content shortly after harvest have increased +b values due to amino acid driven surface interactions/reactions, possibly with carbohydrates; with amino acids likely being the limiting reagents in those cases.

Whether the amino acids' positive correlation with +b and negative correlation with some spectrophotometer color properties was an indication that

amino acids were “leaking” onto the fiber surface during harvest is yet to be seen. One study revealed that across a sample of cottons having the same inter-sample color grading, the color measurements for Rd and +b within each sample showed greater variation within the samples (Cui et al., 2013). If these increased intra-sample variations in +b are due to areas of “spotty” amino acid and/or sugar content, it would likely be more in line with the deposition of those chemicals. The findings of this study definitely point to a noteworthy relationship between surface amino acid content and cotton fiber color ratings, as seen in Figure 6 where the colors of the reacted ninhydrin solution and the cotton color visibly change as the +b rating increases. Whether or not the Maillard reaction is the only pathway taking advantage of this clear relationship is of interest.

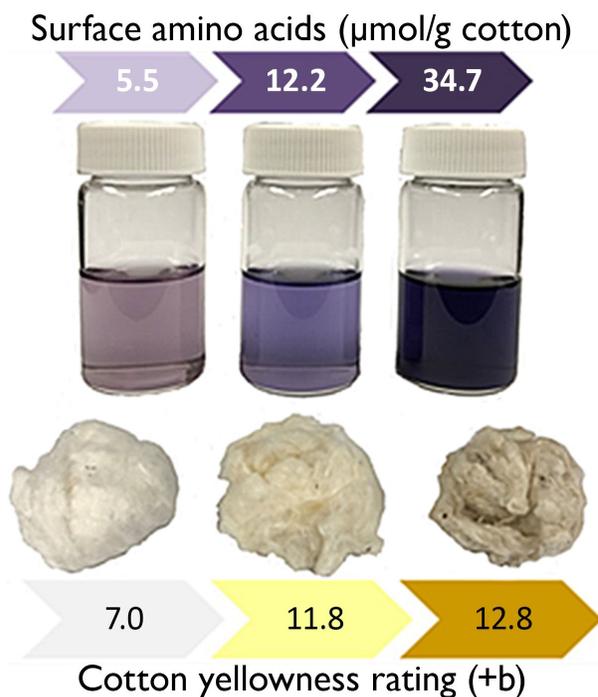


Figure 6: As surface amino acids increase on cotton post-harvest (represented by an increase in the ninhydrin-reacted Ruhemann's purple in solution), there is an increase in the +b cotton rating.

It is important to remember that the ninhydrin test does not account for the presence of proline on the cotton surface fibers; however, the test was an easy, relatively fast way to detect the amine-positive species that facilitate some color-change reactions, such as the Maillard reaction. Ultimately, we want to use these methods to demonstrate quantitative relationships between fiber properties and to determine the results of imposed and natural ageing on

cotton fiber color change. It is imperative to utilize modern ion chromatography for an accurate separation and quantitation of each individual amino acids present on the cotton fiber to discern which amino acid(s) may have the greatest impact on cotton color development and other fiber properties.

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### AUTHOR CONTRIBUTIONS

The corresponding author developed the test design and protocols, lead the research, ran the experiments, and took the lead on writing the paper. The co-authors contributed to writing the paper, obtained/provided samples, and contributed in the development of the test design and experimental protocols.

### CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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### REFERENCES

- Ashraf, U., T. Hussain, and A. Jabbar. 2014. Effect of cotton fiber and yarn characteristics on color variation in woven fabric dyed with vat dyes. *J. Text. I.* 105, 1287-1292.
- Aspland, J.R., and S.A. Williams. 1991. The effect of cotton color grade on the color yield of dyed goods. *Dyeing Cotton.* 23, 23-25.
- ASTM. 2005. Standard test method for measurement of physical properties of cotton fibers by high volume instruments, D5867-05.
- Burks, M.L., and N.W. Earle. 1965. Amino acid composition of upland cotton squares and Arizona wild cotton bolls. *J. Agr. Food Chem.* 13, 40-42.
- Clemens, R.A., J.M. Jones, M. Kern, S.Y. Lee, E.J. Mayhew, J.L. Slavin, and S. Zianovic. 2016. Functionality of Sugars in Foods and Health. *Compr. Rev. Food Sci. Food Saf.* 15, 433-469.
- Cui, X., Y. Cai, J. Rodgers, V. Martin, and M. Watson. 2013. An investigation into the intra-sample variation in the color of cotton using image analysis. *Tex. Res. J.* 84, 214-222.
- Ellsworth, P.C., R. Tronstad, J. Leser, T.J. Henneberry, D. Hendrix, D. Brushwood, S.E. Naranjo, S. Castle, and R.L. Nichols. 1999. Sticky Cotton Sources and Solutions [Online] Available at <https://www.cottoninc.com/wp-content/uploads/2015/12/StickyCottonSourcesSolutions.pdf> (verified 10 Sept. 2018).
- Elmore, C.D., and H.R. Leffler. 1976. Development of cotton fruit. III. Amino acid accumulation in protein and nonprotein nitrogen fractions of cottonseed. *Crop Sci.* 16, 867-871.
- Friedman, M.. 2004. Applications of the ninhydrin reaction for analysis of amino acids, peptides, and proteins to agricultural and biomedical sciences. *J. Agric. Food Chem.* 52, 385-406.
- Gamble, G.R. 2007. The effect of bale ageing on cotton fiber chemistry, processing performance and yarn quality. *J. Cotton Sci.* 11, 100-103.
- Gamble, G.R. 2008. Method for the prediction of the rate of +b color change in upland cotton (*Gossypium hirsutum* L.) as a function of storage temperatures. *J. Cotton Sci.* 12, 7. 2008-2014.
- Ghosh, A., S. Das, and A. Majumder. 2015. A statistical analysis of cotton fiber properties. *J. Inst. Eng. (India): Series E* 97, 1-7.
- Hequet, E.F., and N. Abidi. 2006. Sticky Cotton. Texas Tech University Press.
- Kazama, E.H., F.M. Ferreria, R.P. da Silva, A.R.B. da Silva, and D.A. Fiorese. 2015. Multivariate analysis of fiber characteristics of dense cotton in different harvest systems. *Aust. J. Crop Sci.* 9, 1075-1081.
- Maliyakal, J.E., and G. Keller. 1995. Characterization of mRNA for a proline-rich protein of cotton fiber. *Plant Physiol.* 108, 669-676.
- Mitchell, R., C.M. Carr, M. Parfitt, J.C. Vickerman, and C. Jones. 2005. Surface chemical analysis of raw cotton fibres and associated materials. *Cellulose.* 12, 629-640.
- Peralta, D.V., C.A. Fortier, C.D. Delhom, J. E. Rodgers, and D.P. Thibodeaux. 2016. Separation and quantitation of plant and insect carbohydrate isomers found on the surface of cotton. *AATCC J. Res.* 3, 13-23.

- Rathert, G. 1983. Effects of high salinity stress on mineral and carbohydrate metabolism of two cotton varieties. *Plant Soil*. 73, 247-256.
- Rodgers, J., G. Schild, X. Cui, and C. Delhom. 2013. Portable color spectrophotometer measurements of cotton color in remote locations. *J. Cotton Sci.* 17, 202-211.
- Thibodeaux, D., J. Rodgers, J. Campbell, and J. Knowlton. 2008. The feasibility of relating HVI color standards to CIELAB coordinates. *AATCC Rev.* 44-48.
- Wakelyn, P.J., N.R. Bertoniere, A.D. French, D.P. Thibodeaux, B.A. Triplett, , M.A. Rouselee, W.R. Goynes, J.V. Edwards, L. Hunter, D.D. McAlister, and G.R. Gamble. 2007. *Cotton Fiber Chemistry and Technology*. CRC Press Taylor & Francis Group, Boca Raton, FL.
- Xu, B., C. Fang, R. Huang, and M.D. Watson. 1998. Cotton color measurements by an imaging colorimeter. *Tex. Res. J.* 68, 351-358.
- Yemm, E.W., E.C. Cocking, and R.E. Ricketts. 1955. The determination of amino-acids with ninhydrin. *The Analyst*. 80, 209-214.