

Biological Contamination Parameters of Cotton Lint as Biomarkers for Fibre Quality; A Preliminary Study

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Abstract: It has been reported for several decades that microbes, which naturally contaminate cotton fibres during crop growth and subsequent storage can have an adverse effect on the structural quality of cotton lint. Although several studies have analysed the relationship between numbers of Gram-negative bacteria or bacterial endotoxin and particular physical properties, these studies have been limited to cotton from the United States, and the possible effects of fungal contamination have not been examined in detail. This study quantified the Gram-negative bacteria and fungal cells, as well as measuring concentrations of bacterial endotoxin and fungal glucan, on cotton lint samples from international sources. Spearman's rank correlation coefficients calculated between these results and quality data analysed by an automated testing instrument revealed several significant correlations. Findings included inverse correlations between the biological contamination parameters and fibre elongation, micronaire and reflectance. The possible causes and implications of these findings were also discussed.

Keywords: Cotton fibres, HVI, Endotoxin, Glucan, Quality

Introduction

Cavitoma is a term that describes damage to cotton fibres caused by contaminating microbes such as bacteria and fungi [1]. This was first investigated over 50 years ago [2] but was at this time, limited to the qualitative presence of micro-organisms and visual assessment of fibre damage by microscopic examination of fibre walls. Since then, higher levels of Gram-negative bacteria have been frequently associated with fibres of poorer quality [3,4]. More specifically, several studies analysing particular fibre properties with automated test instruments, have found significant correlations between Gram-negative bacteria and fibre length, strength, micronaire and yellowness [5-7]. These structural parameters have also been found to correlate with bacterial endotoxin concentrations on cotton lint [8]. Since endotoxin is a chemical produced by bacteria, which is utilised as a biomarker to indicate total Gram-negative bacterial biomass, this also implies that fibre quality is related to the presence of bacteria. Table 1 highlights data from previous studies, which demonstrated significant negative correlations between endotoxin concentrations and all fibre parameters except colour, which showed positive correlations.

However, the correlations observed in these studies are not consistent, and although the majority are statistically significant, some of the coefficients are low. Further to this, these and other studies regarding the effects of Gram-negative bacteria or endotoxin on cotton quality are largely only descriptive, giving very little explanation of the reasons underlying the reported correlations. Due to the complexities surrounding some of the fibre properties studied, more focused evaluation is

required to assess these effects definitively.

Fibre damage reportedly occurs when microbial contamination decreases the integrity of the thin cuticle and primary wall. Hence, in addition to bacteria, fungal cells may also contribute to reduced cotton quality. Several fungi previously isolated from cotton are cellulolytic, i.e., they produce the enzyme cellulase, which breaks down cellulose, and could potentially damage the primary and secondary cell walls of the fibres [10,11]. The majority of previous studies investigating correlations between cotton biological contamination and specific fibre quality parameters have focussed on Gram-negative bacteria or endotoxin on US cottons. Therefore, it was of interest to examine whether these relationships extend to cottons from diverse sources and whether fungal cell counts, or glucan levels as a biomarker of total fungal contamination,

Table 1. Correlation coefficients of endotoxin content and Gram-negative bacterial counts paired with various cotton fibre properties from different studies

Property	Correlation coefficient			
	Gram-negative bacteria		Endotoxin content	
	Fischer <i>et al.</i> [5]	Morey <i>et al.</i> [6]	Morey <i>et al.</i> [8]	Morey <i>et al.</i> [7]
Greyness	0.06	0.08	0.02	0.59*
Length	-0.46*	-0.55*	-0.42*	-0.18
Uniformity	-0.53*	-0.45*	-0.38*	-0.14
Micronaire	-0.71*	-0.50*	-0.37*	-0.23*
Strength	-0.36*	-0.42*	-0.32*	0.01
Yellowness	0.61*	0.56*	0.51*	0.23*

*Denotes significant correlation at $p = 0.1\%$ level, adapted from Sasser [9].

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demonstrate the same patterns. Methods employed to reduce the presence of organisms that cause the deterioration of cotton fibres, could also help decrease the risk of industrial exposure to endotoxins and glucans, which can lead to respiratory inflammation and the lung disease byssinosis [12], hence the benefits may be two-fold.

Materials and Methods

Materials

Equipment

All structural quality testing facilities and equipment were provided by the International Cotton Association (ICA), through the Liverpool Cotton Research Corporation, at the Cotton Exchange Building, Liverpool, UK. A high volume instrument (HVI) (Uster Technologies, Switzerland) was used to measure fibre structure parameters. The instrument was calibrated using United States Department of Agriculture (USDA) standard cottons (for analysing length, strength, elongation and micronaire) and a series of ceramic tiles (for analysing colour components). Biological contaminant analyses were performed at the Welsh School of Pharmacy, Cardiff, UK.

Cotton Samples

Thirteen cotton lint samples were provided by the International Cotton Association (ICA), Liverpool, UK. Samples were from Benin, China (2 samples), Iran, Ivory Coast, Paraguay, Syria, Tajikistan, Turkey, USA, Uzbekistan, Zambia and Zimbabwe, all were harvested in the 2001/2002 season and analyses were carried out within 12 months of sample receipt. The samples represented a range of each structural parameter. Upper half mean length and length uniformity varied from 1.057 to 1.478 inches, and from 80.7 to 89.7 % respectively, strength data varied from 24.9 to 36.8 grams per tex, elongation from 5.4 to 8.4 %, micronaire values ranged from 3.24 to 4.75, Hunter's reflectance (Rd) and yellowness (+b) ranged from 60.7 to 80.9 %, and from 7.9 to 12.6 respectively.

Methods

Automated Instrument Analysis

Cotton lint samples of 80-100 g were stored in the ICA arbitration room at 20 °C prior to testing. Samples were analysed for moisture content to ensure they were all within the range 6.75-8.25 % prior to evaluation. A fully automated testing instrument (HVI) was utilised to measure upper half mean length, length uniformity, micronaire, strength, elongation, reflectance (Rd), and yellowness (+b) of cotton fibres (technical information relating to these parameters is available elsewhere [13]). All measurements were performed in triplicate, with the results recorded simultaneously on a linked computer

using HVI software (Uster Technologies, Switzerland).

Measurement of Biomarkers

All determinations were made on replicate (six) sub-samples of cotton fibre. Each sub-sample was removed from a different location within the main sample, in order to establish the mean of a cross section of fibres that would have originated from different cotton plants. This procedure was adopted to ensure the samples were as representative as possible of the bale of origin. The measurement of Gram-negative bacteria was performed by extracting bacteria from 0.6000 g (± 0.0005 g) cotton lint into 10 ml phosphate-buffered saline (0.01 M, pH 7.4) by shaking on a vortex multi-mixer at 1000 rpm for 60 minutes, followed by dilution spread-plating as described previously [14]. Endotoxin concentrations were assessed by similar extraction and application of the *Limulus* amoebocyte lysate (LAL) endotoxin assay, as described elsewhere [15].

Fungal cells were extracted as for Gram-negative bacterial cells (techniques adapted from Krysińska-Traczyk *et al.* [16]). Malt extract agar with added chloramphenicol (30 $\mu\text{g}/\text{ml}$) was used as the growth medium (techniques adapted from Fisher and Sasser [17]); agar plates were prepared and spread plating procedures were carried out as for bacterial cells. Plates were inverted and incubated at 25 °C (± 2 °C) for three days, followed by manual viable cell counting. Glucan measurements were performed using an adapted LAL assay applied to supernatant prepared by shaking 0.2000 g (± 0.0005 g) cotton lint into 4 ml pyrogen free water on a vortex multi-mixer at 1500 rpm for 60 minutes. Glucan levels were attributed to the difference between endotoxin-specific (with added endotoxin-specific buffer) and non-specific (minus the buffer) LAL assays (adapted from Krajewski *et al.* [18]).

Statistical Analyses

Statistical analyses were carried out using the SPSS statistical software package (Version 12 for Windows). Relationships between two data sets were calculated by Spearman's rank correlation analysis ($n=13$).

Results

Analyses of cotton samples revealed a range of Gram-negative bacterial numbers ($216830 \pm 30413 - 713 \pm 212$ CFU/g), endotoxin concentrations ($137.89 \pm 21.55 - 8.30 \pm 0.89$ ng/g), fungal cell counts ($9250 \pm 820 - 281 \pm 29$ CFU/g) and glucan levels ($2964.42 \pm 313.90 - 15.96 \pm 5.18$ LAL reactive units/g). Statistically significant rank correlations were found between contamination levels and three structural parameters; elongation (correlated with endotoxin concentrations and fungal counts), micronaire (correlated with endotoxin and glucan measurements) and reflectance (correlated with endotoxin and glucan data). All significant correlations were inverse relationships (Table 2).

Table 2. Spearman's rank correlation coefficients of biological contamination parameters and HVI data for thirteen cotton lint samples from diverse countries of origin

Property	Correlation coefficients			
	Bacteria	Endotoxin	Fungi	Glucan
Elongation	-0.522	-0.582*	-0.555*	-0.412
p value	(0.067)	(0.037)	(0.047)	(0.162)
Length	-0.225	-0.302	-0.071	-0.253
p value	(0.459)	(0.316)	(0.817)	(0.405)
Length uniformity	-0.451	-0.538	-0.121	-0.368
p value	(0.122)	(0.058)	(0.694)	(0.216)
Micronaire	-0.214	-0.571*	-0.368	-0.659*
p value	(0.482)	(0.041)	(0.216)	(0.014)
Reflectance	-0.505	-0.764**	-0.275	-0.549*
p value	(0.078)	(0.002)	(0.364)	(0.049)
Strength	0.033	-0.302	-0.214	0.181
p value	(0.915)	(0.316)	(0.482)	(0.553)
Yellowness	0.467	0.203	0.176	0.231
p value	(0.108)	(0.505)	(0.566)	(0.448)

*Denotes statistical significance ($p < 0.05$), **denotes statistical significance ($p < 0.01$), significant correlations are displayed in bold font and p values in parentheses.

Discussion

In order to assess the impact of the biological contamination of cotton fibres on structural quality, viable (i.e. living) bacterial and fungal cells were isolated from cotton fibre samples and quantified within twelve months of sample receipt. However, numbers of viable microbial cells do not remain stable on cotton lint following harvest [19], and therefore may not be a reliable indicator of total contamination levels. In light of this, concentrations of endotoxin and glucan, chemicals that originate from Gram-negative bacteria and fungi respectively, but which retain biological activity after the death of their source cells [20,21], were also measured and analysed for correlations with quality parameters. Only three of the seven structural parameters demonstrated significant correlations with levels of biological contamination, these were fibre elongation, micronaire, and reflectance.

Fibre elongation was inversely correlated with endotoxin concentrations and fungal cell counts. This indicates that fibres with high numbers of total Gram-negative bacteria (represented by endotoxin levels) or fungal cells have reduced elasticity, leading to associated problems such as higher end-breakages during spinning and lower productivity due to greater wastage of raw material. This was particularly notable as fibre strength itself demonstrated no significant correlation with any contamination parameter measured, which suggests that these two fibre qualities are affected independently. Microbial cells secrete a wide variety of enzymes, as well as a range of chemical products as a result of biological processes

such as fermentation, including acids and alcohols [22]. These compounds could cause damage to the fibre cuticle and be responsible for the decrease in fibre elongation prior to breakage. Levels of endotoxin and glucan were significantly correlated with fibre reflectance values. It is notable that endotoxin concentrations demonstrated a highly significant rank correlation with this data. Again, these were inverse relationships, the consequence being that fibres with high contamination had low reflectance values. This may also be due to the localised build up of microbial products, which dull fibre brightness. However, it should be noted that fibre yellowness was not significantly correlated with any contamination parameter, although the weak correlations demonstrated with yellowness data were all positive, suggesting that to some degree microbial contamination (particularly Gram-negative bacteria which demonstrated a comparatively strong correlation coefficient) might increase the yellow appearance of some fibres.

Micronaire is a more complex issue, represented by the air permeability of a mass of cotton fibres compressed to a fixed volume. A cotton fibre compares to a hollow tube with a central lumen; immature fibres have a thinner wall and can easily collapse to form a ribbon under pressure. Hence, these fibres demonstrate a low air flow and micronaire reading. However, as a fibre matures, increasing layers of cellulose develop in the interior of the fibre, leading to mature, rigid fibres and increased micronaire values. Micronaire data was inversely correlated with both endotoxin and glucan concentrations, this may indicate that fibres with high levels of contamination remain thin and fine, whereas those with low contamination can mature normally. However, it has also been reported that immature cotton fibres (with low micronaire values) have higher levels of free plant sugars than mature fibres [23]. These are simple sugars, including glucose, fructose, and sucrose, which are all excellent nutrition for bacteria and fungi. Higher levels of this food source could support larger numbers of microbes, giving a negative correlation between endotoxin/glucan and micronaire data. Additionally, coarse fibres (having increased micronaire readings) may carry less microbial contamination than finer fibres because they have a lower surface area per gram of cotton [9]. Further research involving standard fibres with well-defined micronaire values would be needed to isolate which of these factors is responsible for the relationship seen.

In addition to the small number of statistically significant correlations in this study, there were also several relatively strong though non-significant correlations, for example between Gram-negative bacteria and elongation, length uniformity, reflectance and yellowness as well as endotoxin concentrations when paired with length uniformity. Hence, only strength and length data remain without any discernable correlation to contamination levels. These findings emerged despite the analysis of a relatively small number of cotton lint samples from very diverse origins, and they are partially reflective of previous results carried out on larger sample numbers of US

cotton fibres. For example, comparable coefficients were demonstrated between Gram-negative counts correlated with yellowness and length uniformity in the current study and the results of Morey *et al.* [6] and between endotoxin concentrations paired with strength [8], fibre reflectance (inverse greyness), and yellowness [7] in other studies. Although the levels of significance varied, perhaps due to the difference in sample number, which was higher in previous studies.

Further investigation in this area is therefore warranted, and controlled studies on artificially contaminated cotton could prove important, as would investigation into the time period over which fibre damage occurs. This may have implications for the storage of cotton prior to processing.

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