

<http://www.pjbs.org>

**PJBS**

ISSN 1028-8880

**Pakistan  
Journal of Biological Sciences**

**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

## Endotoxin Eradication Techniques and Their Effect on Lint Chemical Constituents

Sh.M. Nawaz, Iftikhar Ahmad, Muhammad Nasir and Atif Nisar Ahmed<sup>1</sup>

Department of Fiber Technology,

<sup>1</sup>Department of Microbiology, University of Agriculture, Faisalabad, Pakistan

**Abstract:** Various techniques for the microdust particles (ericlotoxins) eradication from raw cotton were trialed. Autoclave method was found the most suitable with maximum removal of the Byssinosis causative with minimum effect upon the cotton chemical constituents while washing and flash heating affected the constituents significantly.

**Key words:** Textile, Cotton, Enclotoxins (Lipopolysacchandes), Endotoxin eradication techniques, Byssinosis, Cellulose, Protein, Ash, Wax content

### Introduction

Cotton is considered as the most important fibrous cash crop in the world. According to the Economic Survey report of 1998-99, Pakistan is earning 126370 million rupees foreign exchange by cotton and cotton products export. Since cotton textile industries run round the clock by the efforts of technical personnels and hard working labourers of the country for maximum production and development. Therefore, the health maintenance of the textile workers must be a job at priority while there is a serious lungs disease Byssinosis associated with raw cotton. The observation is that raw cotton contains some toxic agents called as endotoxin llipopolysaccharidos i.e., LPS) in the form of microdust particles as it was reported by Tortora (1987) that the cause of Byssinosis is the toxin from bacteria or fungi that is present in raw cotton.

Therefore, continuous inhaling in that toxin contaminated environment, the textile workers become victim of the respiratory disfunctioning, particularly.

Research tends towards the conclusion that microdust, mostly in the range of 1 to 5  $\mu\text{m}$ , carried endotoxin that emommutely impair lung's capacity and lead to Byssinosis (Steadman, 1997). Different techniques were adopted in the pot to reduce the Byssinogenic potential of raw cotton and it is generally agreed that the causatives are water soluble materials. Domelsmith *et al.* (1986) found that the unwashed cotton sample was 87 percent cellulose; washing and bleaching increased the purity of cellulose upto 88-89 percent approximately. Analysis of cotton lint has shown that major constituent of cotton fibre was cellulose (Hamby, 1965). Further it was reported by that medium stapled cottons grown at Faisalabad differed significantly in cellulose content with high range from 85.17 to 93.31%.

Protein and Ash content are found lowered due to washing treatment Domelsmith *et al.* (1986), Only the steaming or autoclaving to raw cotton was thought to be the most suitable method for the removal of endotoxin. This idea came from dapyrogenation of laboratory instrument and glassware. Therefore a comparative study of various techniques for endotoxin removal was conducted for the choice of the best suitable method having minimum effect on cotton constituents.

### Materials and Methods

The present research work was conducted in the Department of Fibre Technology with the co-operation of Department of Microbiology, University of Agriculture, Faisalabad, Central Cotton Research Institute, Multen end Mustafa Spinning Mills, Faisalabad.

Cotton samples of varieties NIAB-78, eliAB-kariehma, FM-682

and FH-634 were collected from Ayub Agriculture Research Institute, Faisalabad.

The following four treatments were conducted on the raw cotton samples for the removal of endotoxin.

- Cotton samples were mechanically cleaned with the help of shirley Analyzer MK-2 according to the instruction laid down in its operation manual. Cotton samples were heated in an electric furnace at temperature 250°C for a time period of 25-30 seconds.
- Cotton samples were autoclaved at temperature 121°C and a pressure of 18 lbs for a time period of half an hour.
- Washing of the raw cotton samples was done according to the procedure suggested by Rousselle *et al.* (1996).

Cotton varieties and endotoxin eradication techniques were used as follows:

Verities: Niab-78 (V1), NIA8-Kanshma (V2), FH-682 1V3 and F11-634 (V4)

Treatments: Mechanical cleaning, Flash heating, Autoclaving and Washing

**Endotoxin analysis:** Endotoxin (Lipopolysaccharides) content was determined by the method as suggested by, Akhtar (1995) and Rousselle and Chun (1995). All glassware was depyrogenated by heating at 180°C overnight. A weighed sample of 2 g was extracted with 200 ml pyrogen-free water by shaking in a glass stoppered flask. The cell suspension were centrifuged (5000 rpm/30 minutes/4°C) and pellets were re-suspended in physiological saline solution containing 0.3% formalin. The cell suspension was stirred continuously on a magnetic stirrer for two days and centrifuged. Each pellet was resuspended in 5 ml sterile phosphate buffered saline (PBS) and mixed well on vortex. All the pellets were pooled and washed three times in sterile PBS by centrifugation.

The packed cells were suspended in 10 ml of distilled water and heated at 68°C in a water bath for 15 minutes.

10 ml of 65°C heated 90% glass distilled phenol was added to the suspension and incubated at 56°C for 10-15 minutes in a water bath.

The suspension was centrifuged (2500 rpm/30 minutes) and aqueous phase (LPS) was cleared off. Phenol by dialyzing against several changes of distilled water at 4°C. Glucose was

considered as a representative of LPS, was measured by calorimetric method (Kolmer *et al.*, 1959).

**Chemical characteristics of cotton**

**Cellulose content:** The cellulose determination was based upon the extraction of all other substances with sulphuric acid and sodium hydroxide according to the method suggested by AOAC (1984).

**Protein content:** The estimation of protein in raw cotton was performed by determining local nitrogen by Kjeldahl's method and than the estimated nitrogen percentage was multiplied by 6.25 (constant factor) to get protein amount, as suggested by AOAC (1998).

**Ash content:** The ash determination was done by burning the cotton samples in open air and then igniting the same in electric muffle furnace at 500°C for not more than 20-30 minutes as recommended by AOAC (1998).

**Wax content:** Wax percentage was determined by extraction of raw cotton fibre with either solvent in a Soxhlet extractor for six hours. The solvent was evaporated and the residue weighed. The percentage was calculated on dry basis of raw cotton as suggested by AOAC (1984, 1998).

**Results and Discussion**

**Cellulose content:** The analysis of data in respect to cellulose content for different cotton varieties under different treatments is presented in Table 1. It shows that the differences in the mean value for varieties and treatments are highly significant whereas their interactions show non-significant effect for cellulose content.

Least significant difference (LSO) test for comparison of individual means indicates non significant increase in cellulose contents under mechanical cleaning and flash heating. It was found that under mechanical cleaning treatments, cellulose content increase from 88.69 to 89.70 percent for NIAB-78, 90.70 to 91.76 percent for NIAEt-Krishma, 88.87 to 90.06 percent for FH-682 and 86.94 to 88.09 percent for FH-634, respectively. This slight gain in cellulose content is because of the removal of non lint substances during mechanical cleaning. Under autoclave, significant increases in the mean values for cellulose contents were observed for varieties NIAB-78 and FK634 whereas non significant increase in the mean values are noted for varieties NIAB-Krishma and F11-682. It is found that autoclave increases the cellulose contents from 88.69 to 90.65 percent for NIA8-78, 90.70 to 91.39 percent for NABKrishma, 88.87 to 90.06 percent for FH-682 and 96.94 to 88.47 percent for FH-634, respectively. Statistically significant gain in cellulose content is observed under washing treatment. It is found that it increases the cellulose content from 88.69 to 93.44 percent for NIAB-78, 90.70 to 94.21 percent for NIAB-Krishma, 88.87 to 94.04 percent for FH-682 and 86.94 to 91.39 percent for FH-634, respectively. Domelsmith *et al.* (1986) found that unwashed cotton contained 87% cellulose while washing and bleaching process increase the purity to approximately 88 to 89 percent. The present results are also supported by the findings of Hamby (1965).

**Protein content:** Least significant difference (LSD) test for comparison of individual means indicated (Table 2) that statistically no change in protein content under mechanical

Table 1: Analysis of variance for Cellulose Content

S.O.V.	df	SS	MS	F. value	Prob.
Variety IVI	3	B6.859	28.953	45.2795	0.0000**
Treatment IT)	4	154.753	39.688	60.5048	0.0000**
VXT	12	4.730	0.394	0.6164	NS
Error	40	25.577	0.639		
Total	59	271.919			

\*\* = Highly significant

Table 2: Analysis of variance for Protein Content

S.O.V.	df	SS	MS	F. value	Prob.
Variety (V)	3	0.045	0.015	1.0185	0,3944E NS
Treatment IT)	4	0.464	0.116	7.8623	11,03:11**
V X T	12	0.009	0.001	0.0482	NS
Error	40	0.590	0.015		
Total	59	1.108			

\*\* = Highly significant N.S. = Non significant

Table 3: Analysis of variance for ASH Content

S.O.V.	df	SS	MS	F. value	Preb,
Variety (V)	3	0.548	0.183	44.2913	0.0000**
Treatment (T)	4	2.205	0.551	884.3587	0.0000**
V X T	12	0.070	0.006	9.4064	0.0000**
Error	40	0.025	0.001		
Total	59	2.849			

\*\* = Highly significant

Table 4, Analysis of variance for Wax Content

S.O.V.	Of	SS	MS	F. value	Prob.
Variety (V)	3	0.081	0.027	44.2913	0.0000**
Treatment IT)	4	0.283	0.071	116.4274	0.0000**
VXT	12	0.018	0.001	2.4237	0.0180*
Error	40	0.024	0.001		
Total	59	0.406			

\*\* = Highly significant, \* = Significant

cleaning, flash heating and autoclave treatments. Significant decrease in the mean values for protein content is observed under washing treatment. It is found that washing lowers the protein content from 1.267 to 1.040% for NIAB78, 1.290 to 1.020% for NIAB-Krishma, 1.287 to 1.020 percent for FH-682 and 1.333 to 1.103% for FH-634, respectively. Similar views were expressed by Rousselle *et al.* (1996) who noted that water extracted some inorganic salts, sugars and proteins from cotton samples.

Domelsmith *et al.* (1986) reported that the nitrogen content of the unwashed sample was 0.16% (1.0% protein), whereas the washed cotton contained 0.03-0.11% nitrogen (0.19-0.69% protein).

**Ash content:** Least significant difference (LSD) test for comparison of individual means (Table 3) indicates that statistically no change in ash content was observed under mechanical cleaning and flash heating treatments. Slight decrease in ash content under mechanical cleaning is because of the removal of sand, dust and other non lint substances. Similar views were expressed by Matthews (1954) who stated that the variation of ash content of cotton fibre might be due to the presence of sand, sail and dust in the cotton. Significant decrease in the mean values for ash contents are observed under autoclave and washing treatments, it is noted that autoclaving lowers the ash content from 1.550 to 1.447% for NIAB-78, 1.350 to 1.257% for NIAB-Krishna, 1.303 to 1.240% for FH-682 and 1.563 to 1.470% for FH-634 respectively whereas washing treatment decreases ash contents from 1.550 to 0.930% for NIAB-78, 1.350 to 0.960%, for NIAB-Krishma, 1.303 to 0.923% for FH.682 and

1.563 to 0.993% for FH-634, respectively. These results confirm the findings of Domelsmith *et al.* (1986) who noted that water washing removed the potassium and other inorganic salts from the cotton fibers causing concomitant decrease in ash.

**Wax content:** Table 4 shows that statistically no change in wax content was observed under mechanical cleaning while lash heating, significantly lowers the wax content. It is found that wax content decreases from 0.4500 to 0.3567% for NIAB-78, 0.4900 to 3833% for NIAB-Karishrna, 0.5700 to 0.4600% for FH-682 and 0.5433 to 0.4400% for FH-834, respectively.

Statistically significant differences in the mean values for wax content are noted under autoclave and washing treatments. It is found that the autoclave method reduced the wax content from 0.45 to 0.38% for NIAB-78, 0.49 to 0.40% for NIABKrishma, 0.57 to 0.47% for FH-682 and 0.54 to 0.47% for FH-634, respectively. Similarly Perkins (1981) observed that the waxy substances on cotton were esters of fatty alcohols of glycerol and had relatively high melting points of 80-85°C. Significant decrease in the mean values for wax content is observed due to washing treatment and is noted that under this treatment, wax content decreased from 0.45 to 0.32 percent for NIAB-78, 0.49 to 0.31% for NIAB-Krishna, 0.57 to 0.33% for FH-682 and 0.54 to 0.32% for FH-634, respectively. These results confirm the views expressed by Perkins (1981) who stated that the more severe washing conditions adversely affected fibre length and removed large quantities of the natural waxes. Whereas the autoclave technique was observed to be the most suitable for considerable removal of endotoxin.

Similarly, Bargeron and Shaw (1985) reported that elevated Weer temperature could affect the natural waxes. Domelsmith *et al.* (1986) stated that the wax content of the cotton lint should decrease with washing, bleaching and scouring.

#### References

AOAC., 1984. Official Methods of Analysis of the Association of Official Analytical Chemists. 14th Edn., Association of Official Analytical Chemists, Virginia, USA., pp: 67-88.

AOAC., 1998. Official Methods and Recommended Practices of AOCS. 5th Edn., Chapter, 4, 5, 12. American Oil Chemical Society, Champaign, IL., USA.

Akhtar, M., 1995. Studied of the immunization of buffalo against boophilus microplus. Ph.D. Thesis, Department of Veterinary Sciences, University of Agriculture, Faisalabad.

Bargeron, J.D. and D.L. Shaw, 1985. Comparison of yarn costs between standard and washed cottons. Text. Res. J., 55: 299-303.

Domelsmith, L.N., R.J. Berni and J.B. Cocke, 1986. Chemical analyses of washed, bleached and scoured and bleached cotton fiber. Text. Res. J., 56: 14-21.

Hamby, D.S., 1965. American Cotton Handbook. Vol. 1. John Wiley and Sons, New York, ISBN-13: 9780470346402, pp: 60-75.

Kolmer, J.A., E.H. Munderloh and M. Smith, 1959. Approved Laboratory Technique. 5th Edn., Appleton Country Crofts Inc., New York, USA.

Matthews, T.M., 1954. Cotton Fiber: Chemical Properties. In: Matthews' Textile Fibers: Their Physical, Microscopic and Chemical Properties, Mauersberger, H.R. (Ed.), John Wiley and Sons, New York, pp: 231-236.

Perkins, H.H., 1981. Effects of washing on fiber properties, dust generation and processing quality of cotton. Text. Res. J., 51: 123-124.

Rousselle, M.A. and D.T.W. Chun, 1995. Endotoxin reduction in dust from heated cotton fibers. Text. Res. J., 65: 501-504.

Rousselle, M.A., J.B. Price, J.A. Thomasson and D.T.W. Chun, 1996. Heat treatment of cotton: Effect on endotoxin content, fiber and yarn properties and processability. Text. Res. J., 66: 727-738.

Steadman, R.G., 1997. Cotton testing. J. Text. Prog., 27: 1-63.

Tortora, P.G., 1987. Understanding Textiles. 3rd Edn., Macmillan Publishing Company, New York, pp: 441-442.