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ORIGINAL RESEARCH

Carmine natural dyeing of chitosan-treated cotton: statistical analysis to enhance energy saving and environmental impact

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Abstract Low molecular weight chitosan was employed for surface modification of cotton fabrics suitable for dyeing with carmine natural dye, without the addition of any coadjuvants into the liquor bath. The dyeing performance was evaluated through the color strength K/S calculated on the dyed fabrics. A full-factorial design of experiments was employed, followed by ANOVA statistical analysis. Two sets of experiments were designed. In the first set the focus was on assessing the influence of chitosan and dye concentration on the color strength (K/S) of the dyed fabrics. The second set of experiments was designed to investigate if shorter dissolution and impregnation times at room temperature would result in acceptable values of color strength K/S, aiming at higher throughput for eventual future industrialization of the process. The results evidenced that it is possible to obtain positive dyeing results with 2% chitosan concentration and 4% owf (on weight of fibers) dye concentration, drastically reducing the chitosan dissolution time and the fabric impregnation time. In addition, the antibacterial activity of selected fabrics was evaluated. The data obtained demonstrated that neither the dyeing process nor the washing severely impacted the antibacterial activity conferred to the fabrics by chitosan.

Keywords Sustainable dyeing · Chitosan treatment · Cotton fabrics · ANOVA statistical analysis · Antibacterial activity

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Introduction

Textile dyeing is one key feature of the textile industry. For centuries, textile substrates have been colored with natural dyes extracted from plants and animals. With the appearance of synthetic dyes on the market, however, natural dyes were slowly replaced with synthetic dyes that show good fastness, color reproducibility and low cost. On the other hand, industrial development, as well as intensive technological

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advances and human activities, have increased environmental pollution due to the enormous amount of non-biodegradable wastes. Among them synthetic dyes are one major source of ecological contamination because the excess of dye not adsorbed causes wastewaters pollution (Jeorge et al. 2023; Majeed et al. 2024; Wang et al. 2023). However, nowadays, the constraints in the use of pollutant compounds have forced the research to find more ecological options (Abid et al. 2012; Deng and Zhou 2024; Khasim et al. 2024; Lellis et al. 2019) and industries to invest in environmentally friendly solutions, likely also at the expense of economic perspective. For this reason, natural dyes are getting renewed attention. They are biodegradable and renewable resources, extracted from plants and animals. Mohammad et al. report a great interest in the use of natural dyes for both textile research and industrial sectors (Shahid et al. 2013). Natural dyes are promising alternatives to synthetic colorants and they can be applied to many types of fibers, including natural fibers (Amin et al. 2024; Brudzyńska et al. 2023; Huo et al. 2021; Mirki et al. 2024). The homogeneity and dye fastness, however, greatly depend on the process conditions as well as the fiber and dye type (Belino et al. 2023; Nambela 2023; Pizzicato et al. 2023). Different animal fibers are more easily dyed with natural colorants thanks to their chemical structures that can link anionic and cationic groups. In particular, many studies dealt with alternative eco-friendly dyeing processes for silk and wool (Lin et al. 2022; Haddar et al. 2018; Tang et al. 2024). On the other hand, cellulose fibers, like cotton, require different mordanting compounds and techniques to achieve good color efficiency. The use of metal-based mordants is often necessary (Emam et al. 2023; Otaviano et al. 2023). Research has focused on using alternative natural molecules, like nettle and shellac, that were employed to dye cotton (Brudzyńska et al. 2023).

Carmine or Cochineal is a natural dye that belongs to the naturally occurring phenolic compounds in the class of anthraquinones. The pigment derives from Carminic acid and is extracted from the female *Dactylopius coccus* insect that colonizes the cactus plant. It is already largely used in the food and cosmetic industry thanks to its safety (Pasdaran et al. 2023). From a market survey (Singh 2024), cochineal natural dye usage had an annual growth of 9.3% in 2023. In the near future, the use of Cochineal dye is expected to grow further in many industrial applications from cosmetics, food, pharmaceutical and also in textiles. In the textile sector, Cochineal has already been employed in the dyeing of silk fabrics mordanted with the natural mordants quercetin, lawsone, and tannin (Amin et al. 2020). It was also used to dye woolen yarn carpets to obtain brilliant and intense reddish colors (Ammayappan et al. 2016). Nevertheless, cellulosic fibers are not easily dyed with carmine dye. In aqueous solutions, cellulosic fibers are negatively charged due to the hydrolysation of their hydroxy groups. For its chemical structure, carmine is negatively charged as well in water, so repulsion between cotton and the natural dye occurs. Mordants are required to overcome the problem.

Among natural resources, chitosan and its derivatives are very promising compounds that can be used in numerous applications. Chitosan is a linear polysaccharide-based biopolymer derived from chitin and extracted from the exoskeleton of crustaceans. Chitosan is produced by deacetylation of chitin through the hydrolysis of the acetamide groups in a strong alkaline environment (Li et al. 2024). It is bio-compatible, biodegradable, and in particular, it possesses antibacterial properties. There is a large number of chitosan applications from the pharmaceutical and biomedical sector (Rostamitabar et al. 2022), cosmetics, food (Amorim et al. 2024) and also in the textile field (Hamida et al. 2024; Hasan et al. 2022; Rahman et al. 2023; Stegmaier et al. 2008; Thambiliyagodage et al. 2023; Verma et al. 2022).

Market research from Chaudhary (2024) reports that the chitosan market industry is expected to increase with a compound annual growth rate (CAGR) of 15.5% from 2024 to 2032. The awareness for environmental preservation has forced both industries and consumers to employ and use products derived from natural and sustainable resources. In the textile sector, chitosan production is likely to expand as well and may stimulate government support. In addition to these peculiarities, chitosan has health benefits thanks to its antibacterial properties. In this contest, chitosan is primarily utilized as an antibacterial finishing of textiles (Grgac et al. 2020; Ullah et al. 2019). The antibacterial activity is displayed thanks to the cationic amino groups of chitosan that attract the anionic groups of the bacteria, thus preventing the bacteria from feeding through the cell membrane (Iqbal et al. 2023).

This manuscript presents an eco-friendly alternative to traditional textile dyeing. Low molecular weight chitosan was employed as a pre-treatment of the cotton fabrics, eventually dyed with carmine natural dye. No other additives were introduced to preserve eco-friendliness of the process. In the literature, there is no evidence of cotton dyeing with only the natural dye molecules in the liquor bath. The work, moreover, investigates the influence of each parameter on the dyeing results. Two full-factorial designs of experiments were performed, followed by ANOVA statistical analysis. In particular, chitosan interaction and adsorption on the cotton fabrics were assessed. In the literature, it is reported that chitosan requires a long treatment time if performed at room temperature with cellulosic fibers (Ferrero, Periolatto 2017). Based on the positive results obtained in our previous works focused on chitosan treatment of cotton fibers (Ferrero et al. 2014; Piccioni et al. 2023; Truffa Giachet et al. 2019), in this work we investigated the possibility of reducing cotton treatment time with chitosan, maintaining the advantage of working at room temperature, to emphasize the sustainability of the process, in terms of energy saving.

In addition, the manuscript evaluates the preservation of the antibacterial properties against *E. coli* and *S. aureus* after dyeing and washing through further characterization performed on some selected fabrics because, as shown in the literature, after dyeing the antibaterial properties may be negatively affected (El Sayed et al. 2023).

Materials and methods

Low molecular weight (50–190 kDa) chitosan (deacetylation 83%, viscosity in 1 wt% in 1% acetic acid at 25 °C, 108 cps, Lot # BCCD0403) was purchased from Sigma Aldrich S.r.l. The plain 100% cotton fabrics, 115 g/m^2 were purchased from Ausiliari Tessili srl (Italy). The carmine natural dye (carminic acid) was kindly supplied by Aromata Group Srl (Italy). Its chemical structure is reported in Fig. 1.

Cotton fabric treatment with chitosan

The low molecular weight chitosan was dissolved in a 2% v/v acetic acid water solution. Chitosan concentrations in the solution were 1%, 2%, and 3% w/v, respectively. Initially, the solutions were magnetically stirred for 24 h. Subsequently, dissolution time was gradually reduced to 6 and 2 h. Cotton fabric of 10 g each were impregnated with the chitosan solutions at different times, starting from 12 h and then reducing the time at 5 and 2 h. Afterward, the impregnated cotton fabrics were padded at 90% wet pick up and dried in a fan oven at 95 °C. They were eventually cured at 150 °C for 3 min.

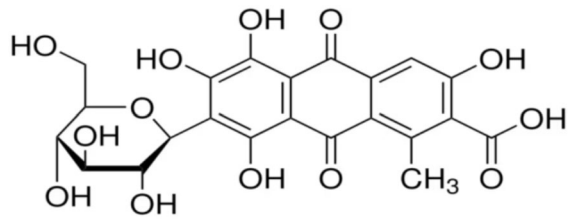

Fig. 1 Carminic acid (natural dye)

Fabric dyeing

The cotton fabrics treated with chitosan were dyed in an AHIBA Nuance Top Speed II equipment. The liquor ratio was set at 1:20 (10 g of fabric in 200 ml bath); the temperature was at 100 °C with a heating rate of 1 °C/min; the process time was 60 min. Speed was 35 rpm and reverse time 3 min. Dye concentration was set at 1%, 2%, 3%, 4%, and 5% owf. As control sample one plain untreated cotton fabric was dyed as well.

Fabric characterization

The chitosan-treated fabric surface morphology was examined with an EVO series (ZEISS) scanning electron microscope with an acceleration voltage of 15 kV, a current probe of 100 pA, and a working distance of 20 mm. The samples were mounted on aluminum specimen stubs with double-sided adhesive tape and sputter-coated with gold in rarefied Argon using a Quorum SC 7620 Sputter Coater with a current of 15 mA for 120 s.

The dyed samples were analyzed with a Datacolor SF 600 X Spectraflesh with CIE standard illuminant D65, 10°. The reflectance values, R, of the dyed samples were measured and the corresponding color strength, K/S, of the fabrics at 520 nm wavelength

was calculated with the Kubelka–Munk correlation, as reported in Eq. (1).

$$\frac{K}{S} = \frac{(1-R)^2}{2R} \quad (1)$$

where K is the absorption coefficient and S is the scattering coefficient.

The washing fastness of the dyed fabrics was evaluated according to the conditions reported in the standard ISO 105-C06. Triton non-ionic detergent water solution was prepared at 4 g/L concentration, the temperature was set to 40 °C and left for 30 min.

Standard ISO 105-D01 was employed for dry cleaning fastness. The fabrics were soaked in Tetrachloroethylene (Riedel de Haën). The temperature was set at 30 °C for 30 min. The results of the washing test were evaluated using the grey scale according to ISO 105-A03.

The tensile strength of the fabrics was determined according to ISO 13934–1:2013 with an electronic universal testing machine Dynamometer Zwick Roell ProLine 5 KN.

The tensile strength was evaluated both in the warp and weft directions.

Antibacterial activity

The antibacterial activity was evaluated on both the chitosan-treated fabrics and the dyed samples. In addition, the antibacterial activity was measured on the washed fabrics. For comparison, the antibacterial activity of pure cotton was evaluated as well. ASTM E 2149–01 method is designed to evaluate the resistance of non-leaching antibacterial treated specimens to the growth of microbes under dynamic contact conditions. The bacteria were *E. coli* ATCC 11229 (Gram negative), *S. aureus* ATCC 6538 (Gram positive). Yeast extract agar and peptone water were supplied by Liofilchem (Italy). The incubated test culture in a nutrient broth was diluted to give a concentration of $1.5–3.0 \times 10^5$ CFU (colony-forming unit)/mL as the working dilution. Each fabric was transferred to a flask containing 50 mL of the working solution. All flasks were shaken for 1 h at 190 rpm. After a series of dilutions, 1 mL of the solution was plated in nutrient agar. The inoculated plates were incubated at 37 °C for 24 h, and surviving cells were counted. The antibacterial activity was expressed in the percent reduction of the organisms after contact with the test specimen, compared to the number of bacterial cells surviving after contact with the control, according to Eq. (2), where A is CFU/mL after contact (end test), and B is CFU/mL at zero contact time.

$$\text{Reduction}\ (\%) = \frac{B-A}{B} \times 100 \quad (2)$$

Statistical analysis

The experiments conducted in this study were designed following a full-factorial plan, wherein all possible combinations of the independent variables were tested. This approach ensures comprehensive coverage of the experimental conditions. Two or more levels were considered for each factor to capture the range of variation within the experimental design.

The experimental results were analyzed using Analysis of Variance (ANOVA), a statistical method commonly employed to assess the significance of differences among group means. By applying ANOVA, we were able to determine the impact of individual factors on the response variable in a statistically rigorous way. In the case of more than two-factor levels, post-hoc tests were carried out to identify any significant differences among them.

Results and discussions

Fabric dyeing

The results of the dyeing process evidenced that the natural dye was successfully fixed on the cotton substrates treated with chitosan. Only the natural dye was added into the dyeing liquor. On the other hand, the cotton fabric that was not treated with chitosan did not show any dye adsorption. Figure 2 displays the reflectance (R%) curves of several dyed fabrics. The graph reports the fabrics with no chitosan treatment and the curves of the samples that evidenced the maximum and minimum reflectance (for both curves dissolution time was 24 h and the fabric impregnation time in the chitosan solution was 12 h).

The curve of the standard white cotton fabrics, not pre-treated with chitosan, shows reflectance values close to 90%. This behavior is associated with the absence of the dye molecules. The fabrics treated

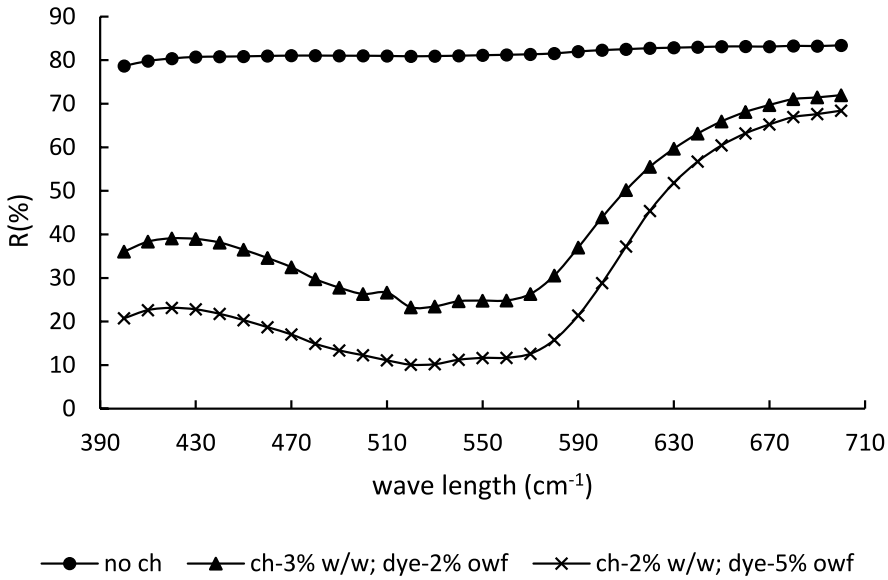

Fig. 2 Reflectance curves of some dyed samples

with chitosan displayed lower R% values, proportional to the dye absorbed in the dyeing process under the conditions described.

The molecules in cellulose are oriented along the fiber axis, with crystalline regions alternating with amorphous regions. Cotton porous structure allows the penetration of water molecules between the fibrils and into the amorphous regions of cellulose. It is also rich in hydroxy groups that confer hydrophilicity to the fibers. Cotton fibers are negatively charged in water. To dye cotton fibers with anionic dyes, like carmine dye, a high amount of electrolytes in the liquor is generally necessary to reduce the repulsion between the negatively charged fibers and the anionic dye, thus promoting dye exhaustion. To avoid the use of salts and large amounts of dye in wastewater, cotton may be cationised. Positively charged sites are introduced on the fibers to improve the substantivity of anionic dyes. By dissolving chitosan in acidic water, the chitosan amino groups are protonated. The protonation leads to the formation of electrostatic interactions bewtween the charged NH_3^+ groups in chitosan structures and the carboxylic and carbonyl groups of the anthraquinone dye as well as of the cellulosic substrates. According to Szadkowski, moreover, the numerous –OH groups in chitosan, Carminic dye and cellulose promote the formation of hydrogen bonding (Szadkowski 2024; Broadbent 2001; Ferrero and Periolatto 2017). A scheme of the interactions is reported in Fig. 3.

In our work, the application of chitosan allowed the dyeing of the cotton fabrics. The decrease in reflectance is associated with the presence of the dye molecules that interacts with the incident light, absorbing the portion of light associated with its wavelength of absorption.

Statistical analysis

The experiments were conducted to examine the influence of the process parameters on the color strength K/S of the dyed samples. The K/S results were measured at 520 nm wavelength. Two sets of experiments, denoted DoE1 and DoE2, were designed.

In DoE1, the focus was on assessing the influence of chitosan concentration and dye concentration on the color strength (K/S) of the dyed fabrics. A full-factorial plan was used. Chitosan concentration (labeled chitosan) and dye concentration (labeled dye) were the fixed factors of the design with 3 levels of concentration for chitosan (1%, 2%, and 3% w/w) and 4 levels of concentrations for dye (2%, 3%, 4%, and 5% owf), as reported in Table S1 of supplementary data.

The chitosan dissolution time in the acetic acid solution (t1) and fabric impregnation time in the chitosan solution (t2) were kept constant at values of 24 h and 12 h, respectively, as suggested in a previous work from Ferrero et al. (Ferrero et al. 2014) who

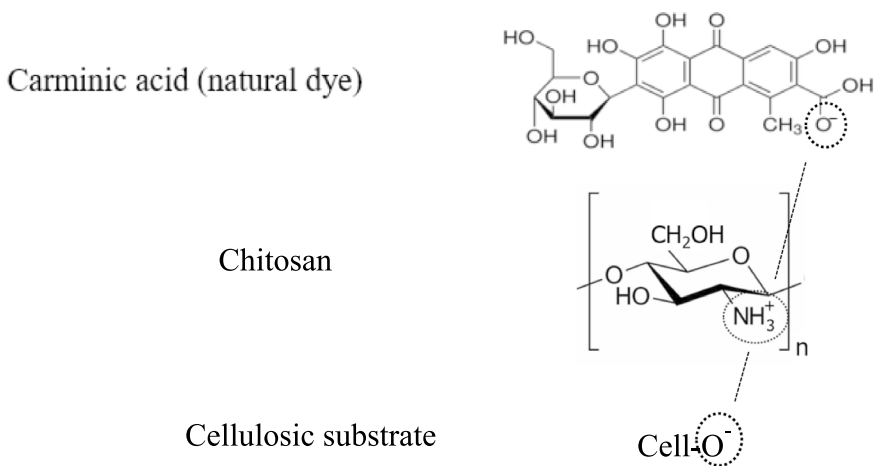

Fig. 3 Interaction scheme of carminic acid, chitosan and the cellulosic substrates in acidic solution

successfully prepared pure cotton gauzes treated with chitosan.

The list of experiments (labeled E1.1–E1.12), along with the K/S response values, is reported in Table S2. The corresponding plots associated with this set of experiments (DoE1), dot plots; main effect plot and interaction plots are reported in Fig. 4.

Figure 4A reports the K/S for the first set of experiments, obtained by varying dye concentration and then chitosan concentration. Figure 4B represents how chitosan concentrations and dye concentrations influence the mean value of K/S obtained in the experiments. Figure 4C plots how the interaction of chitosan and dye concentration influence the mean K/S values of the experiments. These diagrams were generated using IBM SPSS Statistics software tool. The outcome of the experiments was statistically analyzed using a two-way ANOVA, focusing solely on the main effects. Figure 4C shows negligible interaction between dye concentration and chitosan concentration. The main assumptions of ANOVA were verified since the residuals are normally distributed (Shapiro–Wilk test p-value equal to 0.669), and no significant heterogeneity of variance was observed in residual plots. Therefore, it can be concluded that both dye and chitosan factors are significant if a significance level (*alpha*) of 0.05 is considered, as reported in Table 1

The ANOVA test was followed by Tukey's HSD and Bonferroni tests to assess the K/S performance of the different levels via pairwise comparisons.

Regarding the *dye* factor, the K/S response is better for higher levels. The difference between level 4 and level 3 is significant (p-value 0.015 for Tukey's HSD, p-value 0.032 for Bonferroni), while the difference between level 5 and level 4 is only borderline significant (p-value 0.033 for Tukey's HSD, p-value 0.051 for Bonferroni). Therefore, levels 4 and 5 are left as the best candidate levels. This is supported by the fundamentals of the dyeing processes. The higher the amount of dye in the liquor, the deeper the fabric color because the dye concentration gradient between the bath and the textile material favors the migration of dye molecules from the liquor onto the fibers. However, by continuously increasing the dye concentration in the water bath, dye molecules aggregation may occur, causing a lower penetration of dye molecules into the fibers (Youssef et al. 2014).

Regarding the *chitosan* factor, level 2 performs better, but only the difference between levels 2 and 3 is significant (p-value 0.025 for Tukey's HSD, p-value 0.032 for Bonferroni). Therefore, levels 1 and 2 are left as the best candidate levels.

In neutral medium the amino groups of chitosan are not protonated; as a consequence, chitosan is not soluble in water. Acidic conditions are necessary to dissolve chitosan. Acetic acid was employed in our study to prepare a 2% w/v water solution. On the other hand, the two crucial parameters that primarily affect chitosan solubilization are the molecular weight and the deacetylation degree (DD). Low molecular weight chitosan is more easily solubilized. The deacetylation degree is the ratio between glucosamine groups to the overall *N*-acetylglucosamine. It is in the range 0–100%. The DD of the low molecular weight chitosan used in this study, was in the moderate range

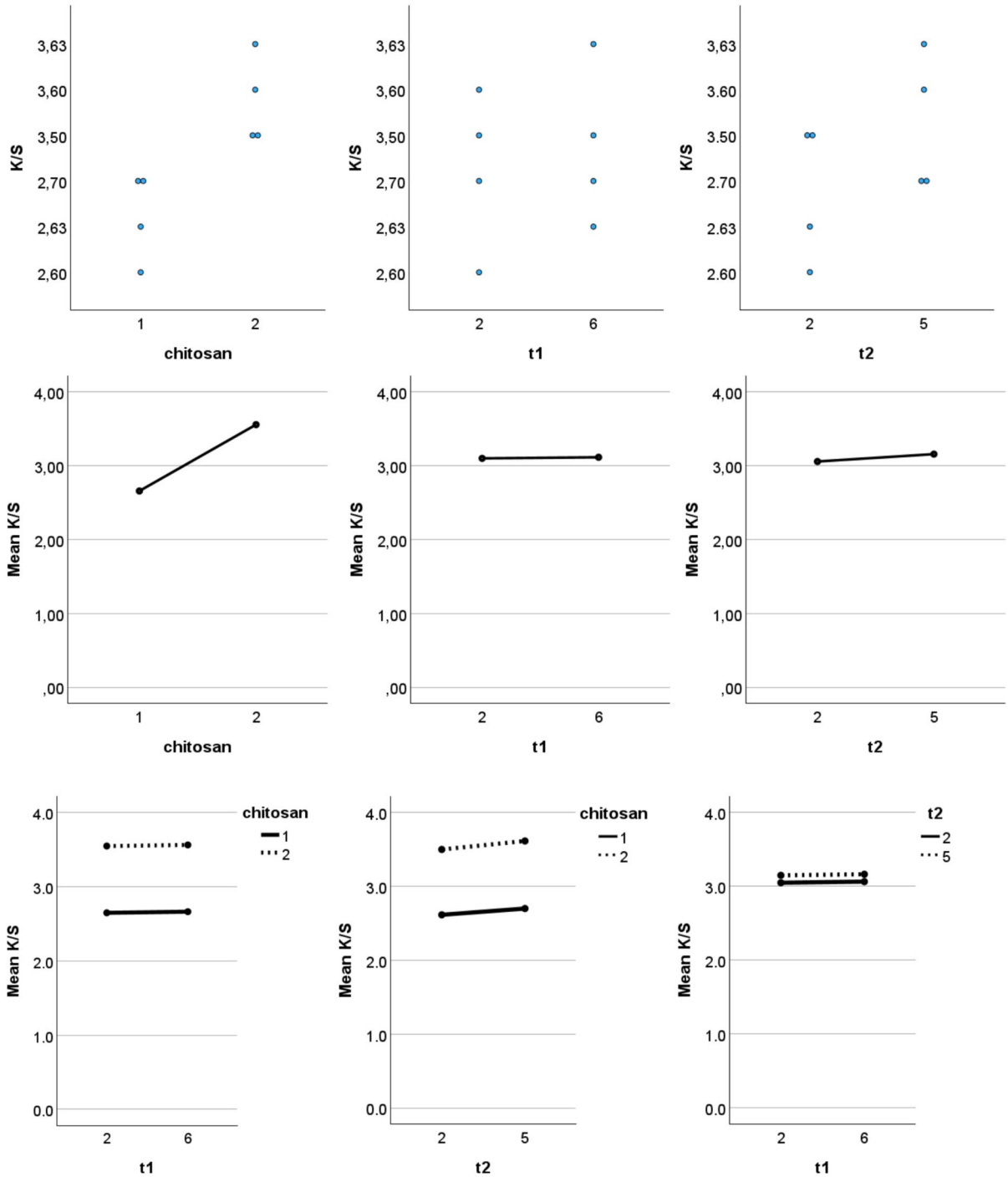

Fig. 4 Plots associated with the first set of experiments (DoE1). **A** Dot plots; **B** Main effect plots; **C** Interaction plot

from 75 to 85%. In this range, chitosan solubility is considered moderate, however, the solution viscosity is high, even at low chitosan concentration (Lv 2016). The pattern of deacetylation, which represents the distribution of the glucosamine and *N*-acetylglucosamine units along the polymer chain, influences

Table 1 ANOVA results for DoE1

Source	DF	Sum sq	Mean sq	F-value	*p*-value
Corrected model	5	9.556	1.911	24.792	< 0.001
Intercept	1	73.954	73.954	959.295	< 0.001
Chitosan	2	1.025	0.512	6.647	0.030
Dye	3	8.532	2.844	36.890	< 0.001
Error	6	0.463	0.077		
Total	12	83.973			
Corrected total	11	10.019			

chitosan dissolution, as well. As reported in the literature (Chattopadhyay and Inamdar 2011), the absorption of chitosan on the textile fibers is affected by the chitosan solution viscosity, which controls the degree of chitosan penetration into the fabric structure. Depending on chitosan molecular weight, a critical concentration causes a dramatic viscosity increase caused by molecular aggregation. This critical concentration increases with decreasing molecular weight, so low molecular weight chitosan is more easily dissolved at higher concentrations. In addition, according to previous literature (Szymańska and Winnicka 2015) chitosan with a block pattern of acetylated and deacetylated units in acidic solutions undergoes aggregation more easily, causing a decrease in chitosan solubility. The chitosan molecular weight used in this work allowed to dissolve 2% chitosan in an acidic water solution. An increment in solution viscosity was observed, increasing the chitosan concentration from 2 to 3% w/v, which may have affected chitosan dissolution and, consequently, chitosan interaction with the textile substrate.

Based on the results of the first set of experiments, a second set of experiments was designed to investigate if shorter dissolution (*t1*) and impregnation times (*t2*) would result in acceptable values of color strength K/S, aiming at higher throughput for eventual future industrialization of the process. Three factors with two levels each were considered, resulting in a 2^3 factorial design. The fixed factors were chitosan concentration (%), chitosan dissolution time (h) in the acetic acid solution (*t1*) and fabric impregnation time (h) in the chitosan solution (*t2*). Levels 1 and 2 for chitosan concentration were chosen to examine in depth their influence on the process, as obtained from the statistical analysis of DoE1. Dissolution time *t1* and impregnation time *t2* were arbitrarily chosen as more cost-effective and energy savings compared to 24 h and 12 h. They were set at 2 and 6 h for *t1*, and 2 and 5 h for *t2* (as shown in supplementary Table S1). In DoE2, dye concentration was kept constant at 4% owf, based on the results of DoE1, which indicated that a concentration of 5% was not significantly better than the value 4%.

The list of experiments (labeled E2.1-E2.8) is reported in Table S3, together with the K/S response values. The corresponding plots associated with this set of experiments, DoE2, dot plots; main effect plot and interaction plots are reported in Fig. 5.

Figure 5A reports the K/S of all the experiments of the second set, obtained by varying chitosan concentration and then varying t1 and t2. Figure 5B represents how chitosan concentration, t1 and t2 influence the mean values of K/S obtained in the experiments. Figure 5C plots the interaction of chitosan and t1, chitosan and t2, t1 and t2 on the mean values of K/S. These diagrams were generated using IBM SPSS Statistics software tool. The outcome of the experiments was statistically analyzed using a three-way ANOVA, which considered only the main effects. Figure 5C shows that the interactions are negligible. The main assumptions of ANOVA were verified since the residuals are normally distributed (Shapiro–Wilk test *p*-value equal to 0.093) and no significant heterogeneity of variance was detected in residual plots

Based on the significance level (*alpha*) of 0.05, it can be concluded that the factors *chitosan* and *t2* are significant, while the factor *t1* is not significant, as reported in Table 2.

Pairwise comparisons between the levels of the significant factors were not performed since only two levels for each factor were considered in the design of experiments.

Regarding the c*hitosan* factor, the second set of experiments demonstrates that level 2 performs better than level 1. Regarding the *t1* factor, level 2 is the best candidate since the factor is not significant and this level would minimize the duration of the process. Regarding the *t2* factor, level 5 is the best candidate. However, the difference compared with level 2 is relatively small.

Finally, an excerpt of the two sets of experiments is presented in Table 3, reporting the best K/S responses for the experiments with c*hitosan* level 2 and *dye* level 4. It is evident how a drastic reduction in total processing time from 36 to 7 h has only a minimal

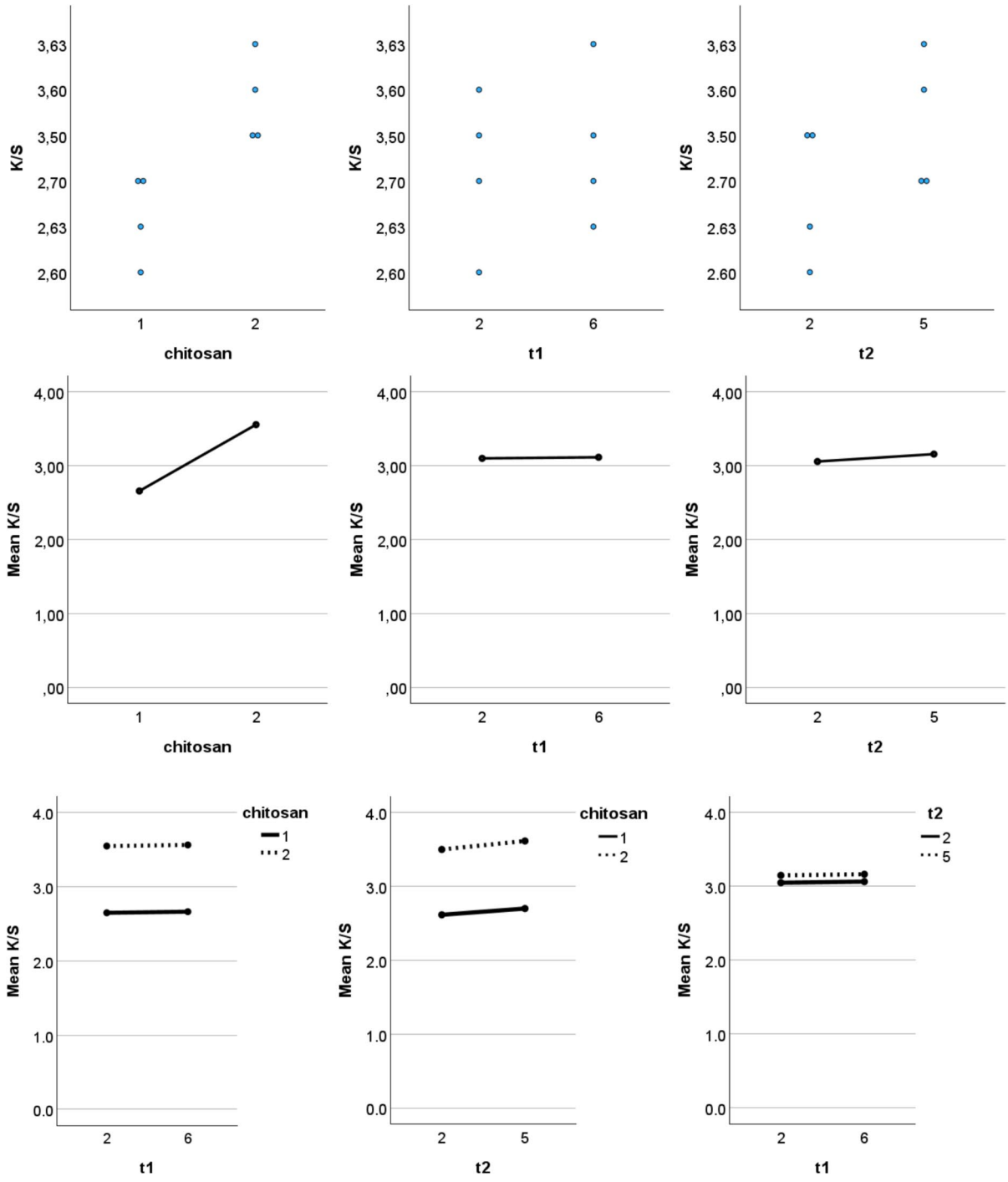

Fig. 5 Plots associated with the second set of experiments (DoE2). **A** Dot plots; **B** Main effect plots; **C** Interaction plot

Table 2 ANOVA results for DoE2

Source	DF	Sum sq	Mean sq	F-value	*p*-value
Corrected model	3	1.64	0.547	2430.296	< 0.001
Intercept	1	77.252	77.252	343,344.222	< 0.001
Chitosan	1	1.620	1.620	7200.000	< 0.001
t1	1	0.000	0.000	2.000	0.230
t2	1	0.020	0.020		< 0.001
Error	4	0.001	0.000		
Total	8	78.894			
Corrected total	7	1.641			

Table 3 Comparison of results from DoE1 and DoE2

Experiment	Chitosan	dye	t1	t2	K/S
E1.7	2	4	24	12	3.63
E2.6	2	4	2	5	3.60

effect on the quality of the results, thus showing the potential for the industrialization of the process.

According to Chattopadhyay (2011), chitosan solution viscosity significantly affects its solubilization and, consequently, its interaction with the textile substrate. In particular, chitosan solution viscosity has a drastic drop in the first 24 h. As soon as the polymer comes in contact with the solvent solution, swelling of the polymer chain takes place and an increase in viscosity is clearly visible. However, by continuously stirring the chitosan solution in the selected solvent, biodegradation of chitosan molecules and hydrolysis of the polymer chain may occur, and shorter molecules cause a reduction of the hydrodynamic volume, thus favoring the polymer chain mobility. Depending on the chitosan concentration and molecular weight, the time to reach the drop in viscosity and chitosan solubilization may vary (Chattopadhyay 2011). In our study it was found that 2% chitosan solution and 2 h stirring was enough to reach satisfactory dyeing results.

Impregnation time *t2* is influenced by solution viscosity, as well. The migration and adsorption of the solubilized chitosan molecules onto the fibers are favored by the high number of amino groups in the chitosan chains that can bond the anionic group of the cotton substrate and, eventually the dye molecules in the next dyeing phase. According to Wang et al. (Wang et al. 2015), three stages are involved in chitosan adsorption onto cotton fibers; the chitosan mass transfer on the cotton fibers surface, the intramolecular diffusion and the final adsorption equilibrium. The authors prepared nanoparticle-sized chitosan and demonstrated that their adsorption onto cotton fibers increases with time. They state that particle size influences the adsorption kinetic. Particles with larger dimensions slow the adsorption mechanism. In our study, commercial chitosan powder was employed. The results evidenced that by increasing chitosan solution contact-time with the cotton fabrics, better dyeing results were reached. However, the slight difference obtained with solution contact times of 5 and 12 h shows that after 5 h the adsorption equilibrium might have been reached.

Fabrics characterization

The cotton fabric treated with 2% chitosan in the initial solution, stirred for 2 h, impregnated for 5 h at room temperature and dyed with 4% owf carmine dye, was chosen for further characterization. Figure 6 shows the images of the chitosan treated and dyed sample, as well as the image of the untreated and dyed cotton fabric, for comparison.

The washing fastness of the dyed fabric was evaluated in non-ionic detergent solution and for dry cleaning. The data obtained demonstrated that the chitosan treatment conferred excellent fastness and no dye adsorption on the sewn reference multi-fiber fabric. The results were evaluated using the grey scale according to ISO 105-A03. It covers a range from value 1–5. Value 5 is associated with excellent fastness, value 1 to very poor fastness. Sample A of Fig. 6 obtained value 4/5 for washing fastness and value 5 for dry cleaning. The strong electrostatic interactions between the dye molecules, chitosan, and the cellulosic substrates are responsible for the durability of the treatment.

SEM analysis was performed on the samples to evaluate the morphology of chitosan treatment and its resistance to washing. Figure 7 reports the micrographs collected. The results show chitosan as a homogeneous layer deposited on the surface of the fibers. Figure 7 C demonstrates that it is not affected by the dyeing process or the washing, confirming the successful grafting of chitosan on the fibers.

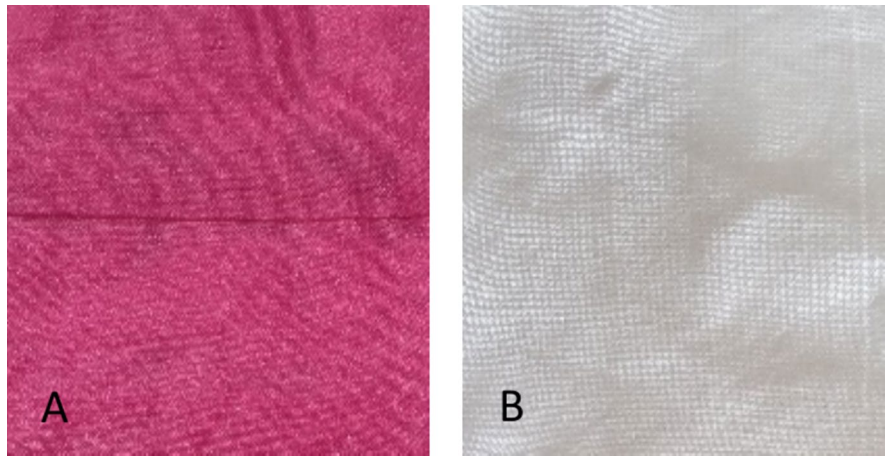

Fig. 6 Cotton fabrics treated **A** and untreated **B** with chitosan and dyed with carmine dye

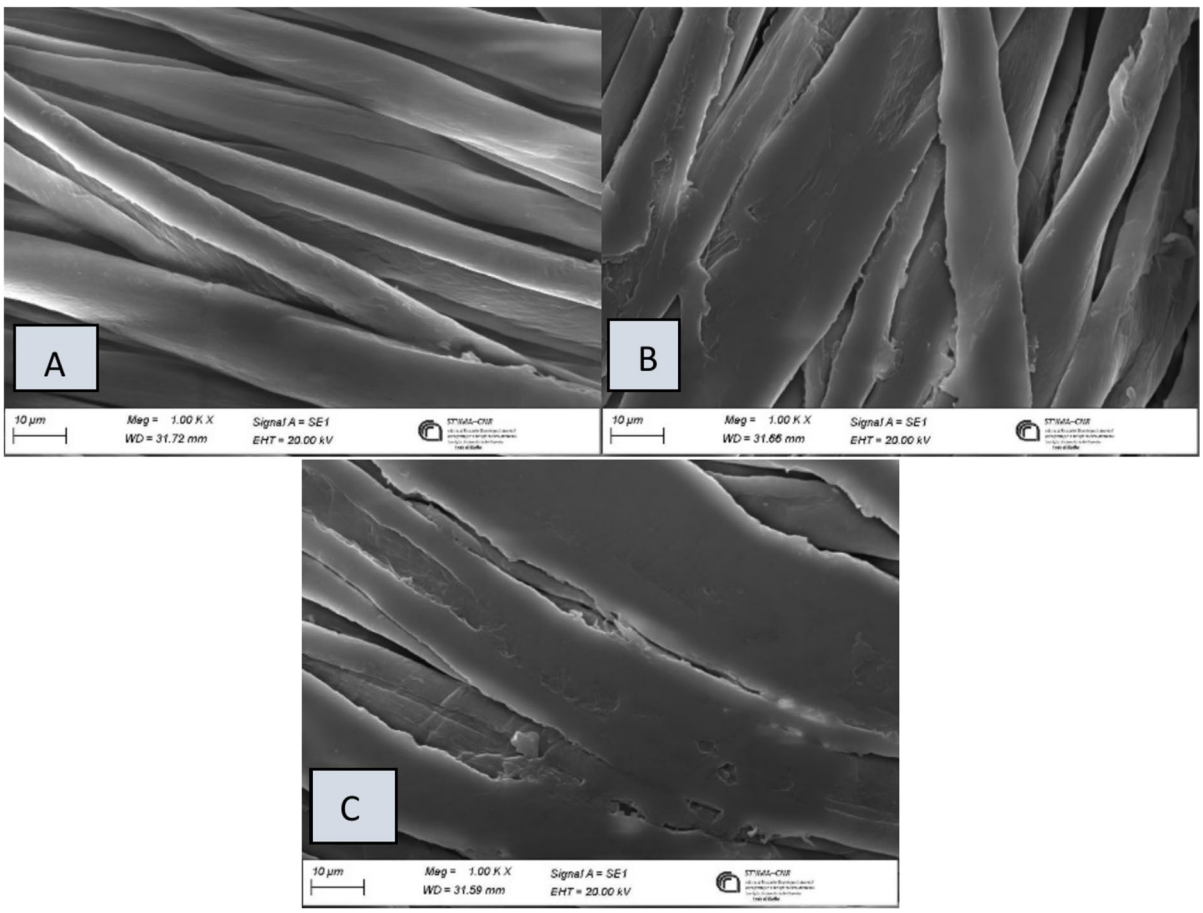

Fig. 7 SEM analysis of the pristine cotton fabric **A** cotton fabric treated with chitosan **B** cotton fabric treated with chitosan, dyed, and washed **C**

Table 4 Mechanical properties of the samples

Sample	Warp		Weft	
	Breaking force (N)	Elongation at break (%)	Breaking force (N)	Elongation at break (%)
Cotton	497 ± 24	6.0 ± 0.2	274 ± 21	23.6 ± 0.2
Chitosan treated cotton	515 ± 20	4.0 ± 0.2	295 ± 22	19.5 ± 0.3
Chitosan treated cotton and dyed	510 ± 21	5.0 ± 0.2	291 ± 23	23.2 ± 0.2
Chitosan treated Cotton, dyed and washed	505 ± 22	5.2 ± 0.3	288 ± 22	22.9 ± 0.2

In Table 4 the mechanical properties of the samples are reported.

The mechanical properties evidenced that chitosan treatment caused a slight stiffening of the plain cotton fabrics, both in the warp and weft directions. Breaking strength of chitosan treated samples is slightly higher if compared to that of plain cotton, while elongation at break is higher for plain cotton. As shown also in the SEM images of Fig. 7, the stiffening of plain cotton is probably due to chitosan deposition onto and between the fibers. In addition the data obtained display that the washing had a negligible effect on the mechanical properties of the fabrics.

Antibacterial activity

Chitosan is known to possess antibacterial properties for both Gram positive and Gram negative bacteria, thanks to its structure, rich in reactive amino groups. The electrostatic interaction between the anionic components of the bacteria cell wall and the cationic nature of chitosan causes the bacteria cell membrane disruption, followed by the inhibition of DNA replication and, consequently, cell death. In particular, according to Thambiliyagodage et al. (2023), the interaction with the anionic structure of teichoic acid in the peptidoglycan layer of Gram positive bacteria induces proteins disruption and cell death. Furthermore, chitosan positive charges are able to neutralize the lipopolysaccharide negative charges of the outer membrane of Gram negative bacteria. Upon outer membrane disruption, chitosan is free to penetrate the cell membrane, leading to cell death (Yan et al. 2021).

In the analysis performed chitosan bacterial activity was evaluated for the Gram positive *S. aureus* and for the Gram negative *E. coli*. Table 5 reports the bacterial colonies percentage reduction after 1 h contact with the test specimen. Three replicates for each sample were evaluated, and the mean value of bacterial reduction is reported in the table.

In Fig. 8 the images of the petri dishes are reported.

The results obtained evidence how the cotton fabric can promote the growth of bacteria. This behavior may cause problems to the fibers that can be damaged and weakened by the bacteria themselves. On the

Table 5 Antibacterial activity of the fabrics

S. aureus	Bacterial reduction%
Cotton	0
Cotton treated with chitosan	100
Cotton treated with chitosan and dyed	98.2 ± 0.6
Cotton treated with chitosan, dyed and washed	86.5 ± 0.6
E. coli	Bacterial reduction %
Cotton	0
Cotton treated with chitosan	100
Cotton treated with chitosan and dyed	100
Cotton treated with chitosan, dyed and washed	91.5 ± 1

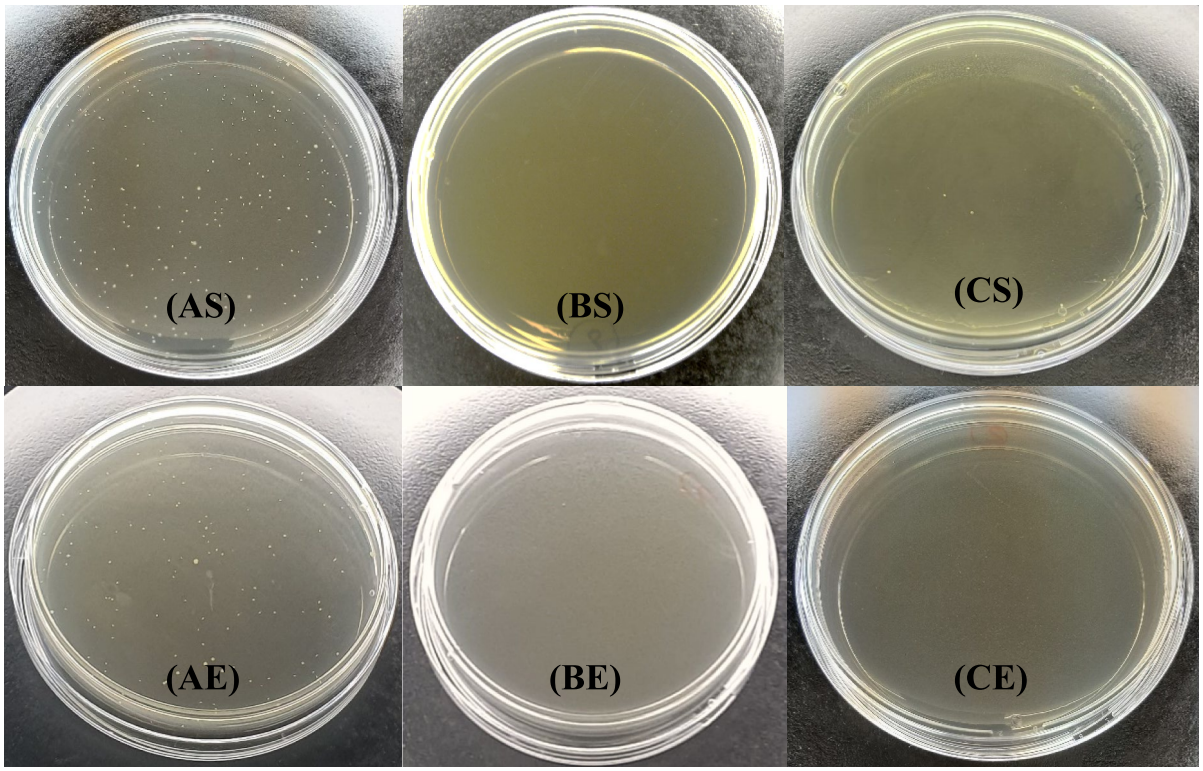

Fig. 8 Petri dishes of the antibacterial test performed for *S. aureus* on pure cotton (AS), cotton treated with chitosan (BS) cotton treated with chitosan and dyed (CS); and for *E. coli* on pure cotton (AE), cotton treated with chitosan (BE) and cotton treated with chitosan and dyed (CE)

other hand, the cotton fabrics treated with chitosan evidenced a drastic bacteria reduction after one hour contact time, for both Gram-positive and Gram-negative bacteria. It is interesting to note that the dyeing process did not affect the antibacterial action of chitosan, and in addition, the washing had minor effects on the biocidal action.

Conclusions

Plain cotton fabrics were treated with low molecular weight chitosan at room temperature. The treatment aimed at cotton dyeing with carmine natural dye. Two full-factorial designs of experiments were performed, followed by ANOVA statistical analysis to investigate the influence of the process parameters on the dyeing results. In particular, chitosan interaction and adsorption on the cotton fabrics were assessed, evaluating the possibility of reducing the processing time, performed at room temperature, without compromising the dyeing results. The statistical analysis showed that the overall processing time could be reduced.

Moreover, it was evidenced that chitosan treatment allows for satisfying dyeing results, reducing the amount of dye used. The dye alone was introduced into the liquor bath without any other additive. The analysis performed on the fabric dyed at some selected process conditions showed that it exhibits antibacterial properties, preserved after washing in non-ionic detergent and dry-cleaning.

Author contributions Peila Roberta: Conceptualization, investigation,writing-original draft. Varesano Alessio: visualisation, writing-review and editing. Vineis Claudia: investigation, writing-review and editing. Tonetti Cinzia: investigation, writing-review and editing. Terkaj Walter:methodology, formal analysis, writing-review and editing.

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Declarations

Competing interest The authors declare no competing interests.

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