sustainability

MDPI

Article

Enhancing Polyphenols and Tannins Concentration on Cotton Dyed with Red Tea

Louise Lambrecht [1,2], Lucía Capablanca [2], Eva Bou-Belda [2], Ignacio Montava [2], Pablo Díaz-García [2] and Jaime Gisbert-Payá [2,*]

[1] Department of Chemistry, HO University College Ghent, Valentin Vaerwyckweg 1, 9000 Ghent, Belgium
[2] Departamento de Ingeniería Textil y Papelera, Universitat Politècnica de València, Plaza Ferrándiz y Carbonell s/n 03801 Alcoy, 03801 Alicante, Spain
* Correspondence: jaigispa@txp.upv.es; Tel.: +34-966528415

Abstract: Natural dyes, obtained from plants, insects/animals, and minerals, are renewable and sustainable bioresource products with minimum environmental impact. However, there are still many issues to solve related to natural dyes; consequently, synthetic dyes are still wildly used. Natural dyes have a low affinity towards the substrate cotton, so a solution had to be found: mordants. Mordants can also be harmful to the environment, which is why bio-mordants are used. The mordant used in this paper is chitosan. Cotton is pre-mordanted using the pad dyeing method. By using the exhaustion method, the fabric was coloured with red *Camellia sinensis* (tea) extracts. The colour, absorption of polyphenols and tannins, and ultraviolet protection (UPF) were tested. A comparison study was carried out between the cotton fabric and the cotton padded with chitosan at two different concentrations. The results are impressive. Cotton pre-mordanted with chitosan can absorb more polyphenols and tannins than cotton itself, and the colour fastness and UPF, once the fabric is laundered, demonstrate there is some kind of bonding between the fibre, quitosan, and active compounds from tea. The UPF was also doubled by using chitosan and the reddish colour obtained by *Camellia sinensis* extracts were darker on the cotton fabric. The increase in UPF protection on mordanted fabrics is higher than the gap obtained by colour difference, which means there are active compounds that do not confer colour, but enhance UPF protection.

Keywords: chitosan; mordant; cellulose; natural dye

Citation: Lambrecht, L.; Capablanca, L.; Bou-Belda, E.; Montava, I.; Díaz-García, P.; Gisbert-Payá, J. Enhancing Polyphenols and Tannins Concentration on Cotton Dyed with Red Tea. *Sustainability* **2023**, *15*, 3062. https://doi.org/10.3390/su15043062

Academic Editor: Zhiping Luo

Received: 30 November 2022
Revised: 4 January 2023
Accepted: 1 February 2023
Published: 8 February 2023

1. Introduction

In recent years, the textile industry developed new colouring technologies to reduce their carbon footprint. Due to the eco-awareness, synthetic dyes were replaced with natural dyes obtained from plants, animals, and minerals [1]. Natural dyes are more environmentally sustainable, compared to synthetic dyes, but their general performance is worse [2]. Synthetic dyes are cheap, can produce a lot of colours, and have a great fastness value. They also have a lot of disadvantages: research stated that some of them can cause allergic reactions, and they even have carcinogenic properties that are harmful for humans and the environment [3].

Natural dyes have a low affinity towards textile substrates, and to improve this, mordants are used. Mordanting is a treatment for textile fabric. Mordants increase the affinity between dye and the fibre. They can be categorised in two groups: heavy metal mordants and natural mordants/bio-mordants. The heavy metals mordants are environmentally harmful; thus, alternatives had to be found [4]. They could form coordination complexes with the natural dye molecule. The coordination improves the stability, but a new environmental problem arises. Metallic mordants offer good results, although, even in algorithm prediction [5], they cause potential pollution and wastewater issues [6]. Extended research had been performed towards bio-mordants. Bio-mordants as citcric acid [7] or chitosan [8]

causes potential pollution and wastewater issues [5]. Extended research had been performed towards bio-mordants. Bio-mordants are derived from different sources, such as plants with a high tannin content, rare earth chlorides (La(III) and Sn(III)), and metal hyperaccumulating plants (example: chlorophyll extracted from plants as a bio-mordants). Another example of bio-mordants is a water-soluble polyphenolic compounds found in wide variety of plant parts, such as wood, fruit, and leaves. Chitosan is used not only as a bio-mordant, but as biocide agent [9].

Natural dyes have recently been widely studied, and their process has been compared with new techniques, such as microwave irradiation [10]. According to the mordant, there are three types of mordanting techniques [11]. The first one is pre-mordanting: the fabric is padded with mordants before the extracted dye solution is applied. The second one is simultaneous mordanting: the fabric is padded with the dye solution and the mordant simultaneously. The third and last is post-mordanting: the fabric is first padded with the dye solution, and after this process, the fabric is padded with mordants.

Chitosan has been used as a bio-mordant for natural fibres, such as wool dyed with cochineal [8], turmeric and madder [12], cellulosic ones (such as linen [13,14] or cotton [15,16]), or even synthetic fibres, such as polyamide [17]. Chitosan has been used as a bio-mordant, in order to improve the colour fastness properties from natural dyes. However, it has also been studied with synthetic ones [18], and it is remarkable that there is a wide number of studies on chitosan and synthetic dyes focused on the removal of those dyes from wastewater [19–21].

Catechin is a kind of polyphenol that cannot link with the cotton fibre effectively unless chitosan is used [22]. Chitosan is [β-(1-4)-2-amino-2-deoxy-D-glucopyranose], and apart from its antibacterial properties [23], it can be used as a mordant on textiles. The chitosan polymer can cross-link with the cellulose polymer in cotton. Generally cross-linking is formed between chitosan and cotton fibre (Figure 1). The link with chitosan increases the total amount of -OH groups. These groups increase the number of H-bonding and, thus, introduce positive amino groups that contribute to the adsorption of reactive dye without salt [24]. The binding of chitosan onto cellulosic fibres is due to ionic and Van der Walls interactions between amino groups from chitosan and hydroxyl groups from cellulose [25]. Another theory suggested by Rippon [26] explains the process as the dehydration of chitosan during dyeing, which implies the removal of amino groups and the chitosan insolubilization with the correspondent imido cross-link with the cellulosic fibres. Li et al. [27] suggested the acid conditions to solve the chitosan in water generate oxycellulose, which enables the reaction with chitosan. The reaction with dyes depends on the nature of the dyeing process. When the pH is alkaline, the hydroxyl groups are the receptors, whilst in acidic conditions, the amino groups are the ones that will react with the dye [25].

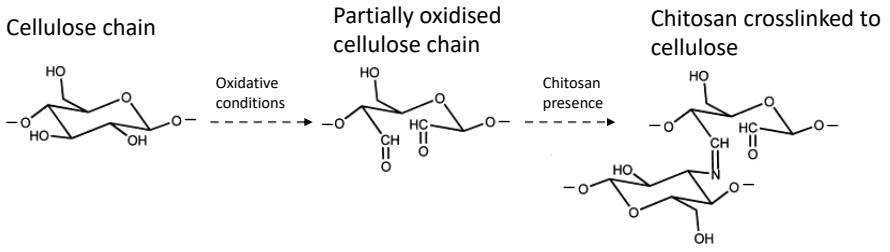

Figure 1. Chitosan cross-linking with cotton fibres.

Tea is comprised of many phytocomponents with active properties such as polyphenols, which decrease the penetration of UV and also decrease the DNA photodamaging [24,25,28,29]. Tea can be used as a natural dye and confer not only colour, but also new properties, such as ultraviolet protection, which is enhanced using mordants, such as chitosan [30]. Determining the polyphenols presence on fabrics can be useful for understanding why polyphenols can help cotton to improve the ultraviolet protection factor (UPF).

The phytoconstituents in tea are generally polyphenols, minerals, vitamins, and amino acids [31]. The number of polyphenols and their types depends on the type of tea. Despite whether the tea (black, white, red, green) comes from *Camellia sinensis* or any other Camellia, the quantity of the constituents differs, as a consequence of the different methods of leaf processing after harvesting. There are many different tea varieties, but green, black, and white are the most common ones, and the differences rely on the level of fermentation. Green tea is a non-fermented one, black tea refers to fermented ones, and white tea is a minimally processed tea. Chinese and Japanese black tea was renamed by the English as red tea, due to the reddish colour from its infused liquid. Among the most important polyphenols, it is worth citing the tannins, both the hydrolysable and condensed ones, and flavonoids [32].

The aim of this paper is to establish a relationship between the liquor content components and the properties that the cotton fabric presents when dyed. Two concentrations of extracts are analysed. The properties are evaluated according to the colour and ultraviolet protection (UPF) that the dyed cotton fabric shows. Wastewaters are analysed in order to determine the phytocomponents presence and their influence on the colour or UPF induced. The influence of chitosan as a bio-mordant, not only for colour, but also for phytocomponents, will be evaluated, and a colour fastness test is addressed, as well.

2. Materials and Methods

2.1. Materials

Every test was conducted with deionised water. Red tea was bought in a local herbalist's shop, from Spanish origin.

To characterise the tea extraction liquor, we used: KIO_3 from R.P. NORMAPUR—VWR internacional S.A.S (Barcelona, Spain); tanic acid ($C_{76}H_{52}O_{46}$) from Sigma Aldrich (St. Louis, MO, USA); HCl 37% from PanReac AppliChen (Darmstadt, Germany); Butanol (BuOH) 99.5% PS, $CH_3(CH_2)3OH$ from PanReac AppliChen; Na_2CO_3 from PanReac AppliChen; Folin–Ciocalteu reactive from PanReac AppliChen; Gailc acid 97% $(HO)_3C_6H_2CO_2H$ (Sigma Aldrich).

2.2. Tea Liquor Extract

The tea extracts were obtained using the infusion procedure. To prepare the red tea extracts, 10 g of red tea was mixed with 200 mL of distilled water. The water was preheated to 50 °C as polyphenols degraded at temperatures higher than 60 °C [33]. Once the temperature reached 50 °C, dried tea leaves were added. Temperature was kept for two hours, as those conditions were optimised previously [34]. Then, leaves were filtered, and liquor was used for characterization and dyeing.

2.3. Pre-Mordanting

In this experiment, the pre-mordanting technique was used. The fabric: 5 g of cotton was immersed in the chitosan solution and brought into the pad dying machine. The chitosan solution was made from 10 g/L of chitosan and 5 mL/L acetic acid. The cotton with chitosan must be put in the oven at 100 °C to evaporate the water for 10 min. Afterwards, the oven is heated to 150 °C for 3 min to secure the linkage from the chitosan with the cotton fibres [35].

The pick-up is one parameter to control—this is the amount of uptake of the mordant by the fabric when it passes through the solution of dye liquor. The rolls pressure was adjusted to make sure the cotton has a high pick-up range. The pick-up was calculated according to Equation (1). The padded cotton with chitosan is dyed.

$$\text{Pick up } \% = \frac{[W_2 - W_1]}{W_1} * 100 \quad (1)$$

W_2 = after padding weight of fabric;
W_1 = before padding weight of fabric;
The cylinder pressure and the speed of the fabrics ensures a pick-up around 80–85%.

2.4. Dyeing: Exhaustion Method

The exhaustion technique means the use of a dye bath of moderately large liquor-to-goods ratio. The fibre is immersed for some time, allowing the dye molecules to attach to the cotton fibres. This method is the most commercial for fabric dyeing [36]. The fabric (5 g) of cotton was put into a dyebath with two concentrations of tea extract (40 and 80 mL) and distilled water till 200 mL, which makes a liquor ratio 1:40. Fabric was kept for 45 min in a hot water bath at 50 °C [34]. The tea extracts were obtained as described before. The liquor ratio is the ratio of the weight of the goods being dyed or processed to the dyebath weight. The fabric absorbs the components from the tea extracts. This was performed for the cotton fabric padded with the chitosan and for the cotton fabric without chitosan as a reference. Every 15 min, a 5 mL sample of the wastewater had to be taken for characterization from polyphenols and tannins.

One bath was prepared without fabric, in order to determine if compounds degraded due to the treatment conditions.

The bath exhaustion was calculated according to a calibration curve prepared with different concentrations of dye extract and measured at λ_{max} = 400 nm.

2.5. Polyphenols Characterization in Wastewater

The Folin–Ciocalteu method [37] was used to obtain the polyphenols in the wastewater of the red tea extracts. The Folin reagent, as purchased, was diluted 10 times. From this dilution, 5 mL was taken and 1 mL of wastewater was added. A total of two milliliters of Na_2CO_3 (75 g/L) were also included in the flask. The mixture was heated to 50 °C for 5 min. The absorbance of the mixture was read at 655 nm. A calibration curve was drawn using a gallic acid solution (80 mg/L, UV–Vis spectrophotometer ZUZI 4251/50 HJD003 supplied by Akralab, Alicante, Spain). The results were expressed in milligram of gallic acid equivalent (mg GAE) per gram of dry tea. Calibration curve was performed with gallic acid 80 g/L (UV–Vis spectrophotometer ZUZI 4251/50 HJD003). Three samples were taken from wastewater and measured. The concentration was obtained from the calibration curve. Then, percentage of reduction in polyphenols was calculated and average value was given. This was performed for the wastewater with cotton and with cotton padded with chitosan.

2.6. Hydrolysable Tannin Characterisation

The hydrolysable tannin content was determined with potassium iodate test, as described by [38]. KIO_3 (2.5% *v*/*v*) was made. This solution was heated for 7 min at 30 °C, then 1 mL of wastewater of the red tea extract was added. The mixture was placed in a water bath and heated to 30 °C for 2 min before putting it into the UV–Vis spectrophotometer. First, a calibration curve had to be obtained by using the tannic acid solution of 5000 mg/L. This solution was carried out by mixing 0.25 of tannic acid with MeOH/H_2O (40/10). Different concentrations of tannic acid were made: 10, 50, 100, 250, 500, 1000, and 1250 mg/L. The absorbance of this mixture was measured by using UV–Vis spectrophotometer ZUZI 4251/50 HJD003 at a wavelength of 500 nm, and a curve was drawn. Three samples were taken from the liquor bath every 15 min. The concentration was calculated using this calibration curve, and the reduction in hydrolysable tannins could be obtained from it. This was performed for the wastewater with cotton and with cotton padded with chitosan. The average value was calculated.

2.7. Condensed Tannin Characterisation

Condensed tannins are also known as proanthocyanidins [39]. Their presence in liquor was determined by means of BuOH/HCl test [40]. This test is conducted as follows: 1 mL of the wastewater of the red tea is added to 5 mL of an acidic ferrous solution (77 mg of $FeSO_4 \cdot 7H_2O$ in 500 mL of HCl/BuOH (2/3). The tubes are heated to 95 °C for 15 min. The absorbance is read at 530 nm (UV–Vis spectrophotometer ZUZI 4251/50 HJD003).

The results were expressed in milligram of cyanidin acid equivalent (Cya) per gram of dry tea. The condensed tannins content was calculated using this Formula (2):

$$\frac{\text{mg CyaE}}{\text{g bark}} = \frac{A \times V \times D \times M \times V_2}{l \times \epsilon \times \nu \times m} \quad (2)$$

where A equals the absorbance at 530 nm; V is the total reaction volume (mL); D is the dilution factor; M equals the cyanidin molar mass (g/mol); V_2 is the aqueous volume extract used to extract the red tea; l is the path length (1/cm); ϵ fits with the molar extinction coefficient 34,700 L/(mol·cm); ν is 0.5 mL and m fits with dry weight mass of tea.

Three samples were taken from the dyeing bath. Using this formula, the concentration of reduction of condensed tannins was obtained. This was performed for the wastewater with cotton and with cotton padded with chitosan. The average value was calculated.

2.8. Colour Characterisation

In order to objectively compare colour difference measurements, the chromatic coordinates (CIE L*, a*, b*) of the CIELAB colour space from the dyed samples were measured. Colour coordinates were obtained using a MINOLTA CM-3600d reflection spectrophotometer (Tokyo, Japan). Specular energy was included. The measurements were made with the standard observer CIE-Lab 10^0 and the standard illuminant D65. On the other hand, the colour difference of the samples was obtained according to the following Equation (3).

$$\text{Colour difference } ((\Delta E^*) = ((\Delta L^*)2 + (\Delta a^*)2 + (\Delta b^*)2)^{1/2} \quad (3)$$

where ΔL* = L* from cotton dyed − L* from cotton + chitosan dyed; Δa* = a* from cotton dyed − a* from cotton + chitosan dyed; Δb* = b* from cotton dyed − b* from cotton + chitosan dyed; "L*" describes the luminosity, "a*" measure of red-green hues, "b*" measure of blue-yellow shades. It should be noted that three measurements were made for each sample, and the average value was calculated.

For the purpose of representing colour surface on treated samples, MINOLTA CM-3600d in above cited conditions was used to obtain the colour strength (K/S).

2.9. Colour Fastness

Colour fastness to washing was performed in accordance with the Standard ISO105 C06:2010 [41]. Two standardised fabrics, one 100% cotton (Co) and another 100 wool (Wo), were used to evaluate the colour discharge. Samples were washed in a Linitest device at 40 °C for 45 min with the recommended soap and flat dried. Lately, when completely dried, they were evaluated for colour discharge and colour degradation.

2.10. Ultraviolet Protection Factor (UPF)

After the colouration of the cotton fibres with or without chitosan, the UPF had to be determined. The UPF testers, also called photometers, will test the UVA and UVB absorption separately. UVA (315–400 nm) has a longer wavelength and is associated with skin aging, UVB (280–315 nm) has a shorter wavelength and is associated with skin burning. The UVA was measured using a lamp as sender and a UVA detector as receiver. This has to be calibrated, and in order to prevent ageing of the lamp or difference in irradiation due to the temperature, the detector must present at 5.6 W/m^2 for the UVA, and UVB can be used at 8 W/m^2 (wavelength per square meter). The lights go through a monochromator, where the bundle of light is divided in different spectrum. The light passes the chromate, through the fabric to the light detector. Certain UVA and UVB waves are blocked by the fabric, and the ultraviolet protective factor (UPF) is a numerical value which represents the degree of protection against UV rays provided by clothing, according to Equation (4).

$$\text{UPF} = \frac{\sum_{\lambda=290}^{400} E(\lambda) * \varepsilon(\lambda) * \Delta(\lambda)}{\sum_{\lambda=290}^{400} E(\lambda) * T(\lambda) * \varepsilon(\lambda) * \Delta(\lambda)} \quad (4)$$

That formula expresses the ratio the amount of time needed to produce damage on the skin protected with textile to the amount of time needed to produce such damage on unprotected skin. The E_λ is the solar irradiance expressed in $\frac{W}{m^2 nm}$, $\varepsilon(\lambda)$ is the erythema action spectrum, λ is the wavelength interval, and $T(\lambda)$ is the spectral transmittance at wavelength λ [42]. Three measurements were made for each sample, and the average value was calculated.

3. Results

3.1. Phytocomponents Presence

Previously, to determine the phytocomponents (polyphenols, hydrolysable, and condensed tannins) presence, the % of dye exhaustion, according to the colour, was determined, as Table 1 shows. An increase in % of bath exhaustion for periods higher than 30 min, and a slight increase when reaching 45 min is clearly appreciated.

Table 1. % Dye exhaustion measured at λ_{max} = 400 nm.

Time (min)	Cotton 40 mL	Cotton + Chitosan 40 mL	Cotton 80 mL	Cotton + Chitosan 80 mL
0	0	0	0	0
15	20.15	15.68	18.75	11.76
30	35.76	39.44	38.94	40.89
45	49.78	52.42	50.46	54.32

Analysis of polyphenols in wastewater when no fabric was placed shows there is a leak around 2.5% of polyphenols, due to the dyeing conditions. When wastewaters from dyeing process are analysed, it is clearly observed from Figure 2a that cotton treated with chitosan shows similar values to those from cotton without chitosan. This means that around 15% of polyphenols have migrated from the bath towards the cotton fibre. However, polyphenols react faster with cotton than with cotton treated with chitosan, and no significative differences can be observed when the concentration of the extract is doubled (40 to 80 mL/200 mL).

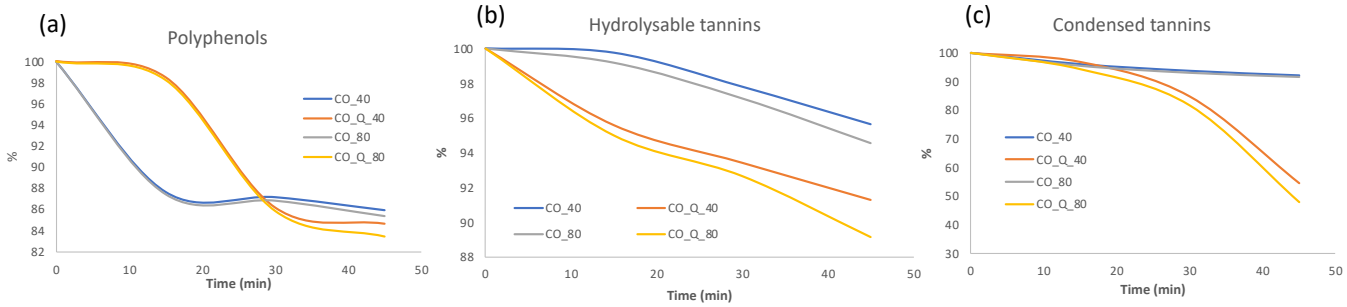

Figure 2. Content of Phytocomponents: (**a**) Polyphenols; (**b**) Hydrolysable tannins; (**c**) Condensed tannins. CO_40: sample dyed at 40 mL/200 mL; CO_Q_40: sample mordanted with chitosan and dyed at 40 mL/200 mL; CO_80: sample dyed at 80 mL/200 mL; CO_Q_80: sample mordanted with chitosan and dyed at 80 mL/200 mL.

Such a quick absorption on the fabric can deal with irregularities in the polyphenol's distribution on the fabric, similar to the phenomena observed when dyeing and the dye is absorbed quickly.

Regarding the hydrolysable tannins, Figure 2b shows the results for cotton and cotton mordanted with chitosan. Those values can be considered as the real ones, as tests conducted on the dyebath without fabric barely showed a leak of 0.5%.

In contrast to what is observed with polyphenols, the speed of hydrolysable tannins is faster for samples treated with chitosan. Similar behavior can be observed when the

dye extract concentration is doubled. Furthermore, the percentage of hydrolysable tannins migrated from bath to fibre is around 5% for cotton and 10% for cotton with chitosan. The mordanted process shows higher values of tannins migrating towards the fibre, and it is almost double than the sample without mordant.

When condensed tannins are evaluated (Figure 2c), the reference flask containing only a liquor bath did not experienced a variation higher than 0.75% of hydrolysable tannins, which enables us to consider that the results show a tolerance around +1%. Focused on the condensed tannins in cotton fabrics, the difference between the cotton and the cotton mordanted with chitosan for both concentrations is noticeable. Wastewater from cotton shows 92% of tannins still remain in the liquor, which implies around 8% has moved toward the fibre. On the other side, mordanted cotton shows a similar behavior to cotton for the first 30 min; however, a considerable drop can be observed when measured at 45 min. Cotton with chitosan reflects a high decrease (54.6%) of tannins in wastewater, which can be translated into approximately 55% of the tannins in cotton. Higher amounts of dye extract imply lower concentration of tannins in wastewater, an effect that is potentiated by the presence of chitosan.

3.2. Colour Characterization

When samples are observed, a clear difference in colour can be noticed. Figure 3 shows cotton fabrics dyed, Figure 3a fits with cotton without dye, whereas Figure 3b is cotton dyed with red tea at 40 mL/200 mL, and Figure 3c belongs to the same cotton but treated with 80 mL/200 mL. Figure 3d,e fit with the mordanted cotton with chitosan and the aforecited concentrations, respectively. Despite the fact that difference is visually evident, the colourimetric coordinates shown in Table 2 offer an objective analysis.

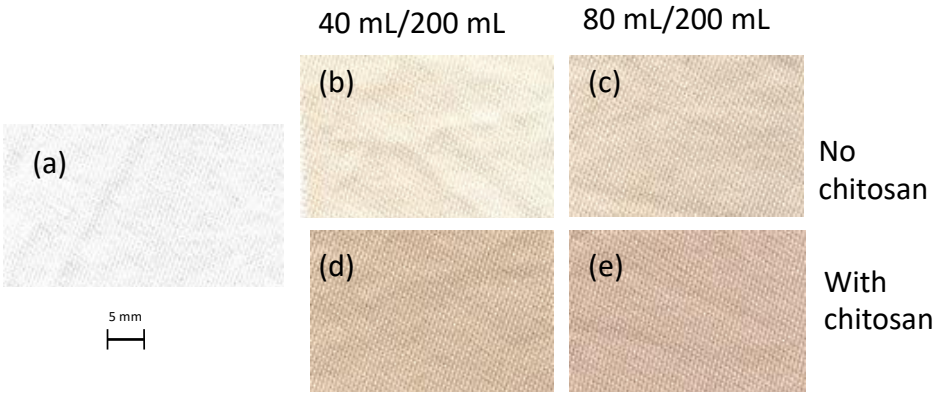

Figure 3. Dyed fabrics with red tea liquor: (**a**) cotton fabric (CO); (**b**) sample dyed at 40 mL/200 mL (CO_40); (**c**) sample dyed at 80 mL/200 mL (CO_80); (**d**); sample mordanted with chitosan and dyed at 40 mL/200 mL (CO_Q_40); (**e**) sample mordanted with chitosan and dyed at 80 mL/200 mL (CO_Q_80).

Table 2. Colour coordinates from dyed samples. CO: cotton without dyeing; CO_Q: CO: cotton mordanted with chitosan without dyeing; CO_40: sample dyed at 40 mL/200 mL; CO_Q_40: sample mordanted with chitosan and dyed at 40 mL/200 mL; CO_80: sample dyed at 80 mL/200 mL; CO_Q_80: sample mordanted with chitosan and dyed at 80 mL/200 mL.

Reference	L	a	b	ΔL*	Δa*	Δb*	ΔE*ab
CO	92.8158	−0.3732	3.9564				
CO_Q	88.4979	−0.4906	3.0713	−4.3179	−0.1174	−0.8851	4.4092
CO_40	82.5565	0.5826	10.8032	−5.9414	1.0732	7.7319	9.8099
CO_40_Q	75.3886	2.9726	15.2524	−13.1093	3.4631	12.1811	18.2271
CO_80	78.5852	3.4205	13.6868	−9.9127	3.9111	10.6156	15.0416
CO_80_Q	72.5898	3.7685	18.9044	−15.9081	4.2591	15.8331	22.8451

Once the cotton fibre is padded with chitosan, the luminosity (L*) decreases slightly, but not noticeably; moreover, a slight increase in b* can be easily appreciated. This means that there is an increase in the yellowness of the fabric due to the chitosan treatment.

When the fibres were coloured with the red tea extracts from the *Camilla sinensis*, the luminosity decreased considerably (see Table 2). This decrease is higher for fabrics treated with chitosan. The cotton fibres and the cotton padded with chitosan has a slightly positive data for a* value, and this indicates the more reddish colour. Every b* value is positive, and this indicates a yellowish touch. Thus, considering that the range for a* and b* is similar, it can be confirmed that it is a brownish colour, as can be observed from Figure 2. The colour difference ΔE between the chitosan-padded fabric and chitosan-padded fabric coloured with red tea extracts is far greater than the cotton fibres coloured with the extracts without the bio-mordant process. There is slight difference between cotton without mordant dyed at 80 mL/200 mL (CO_80) and cotton mordanted dyed at 40 mL/200 mL (CO-Q_40), ΔE*ab = 3.5874).

For the surface colour, it can be useful to observe the colour strength (K/S), as can be seen in Figure 4. The graph shows that there has been a marked rise in colour strength for samples mordanted, in comparison to the same treatment without mordanting. Regarding the concentration of extract used, as was expected, higher concentrations (80 mL/200 mL) are more intense than lower ones (40 mL/200 mL). The results, which are in accordance with the colour differences, are reported in Table 2.

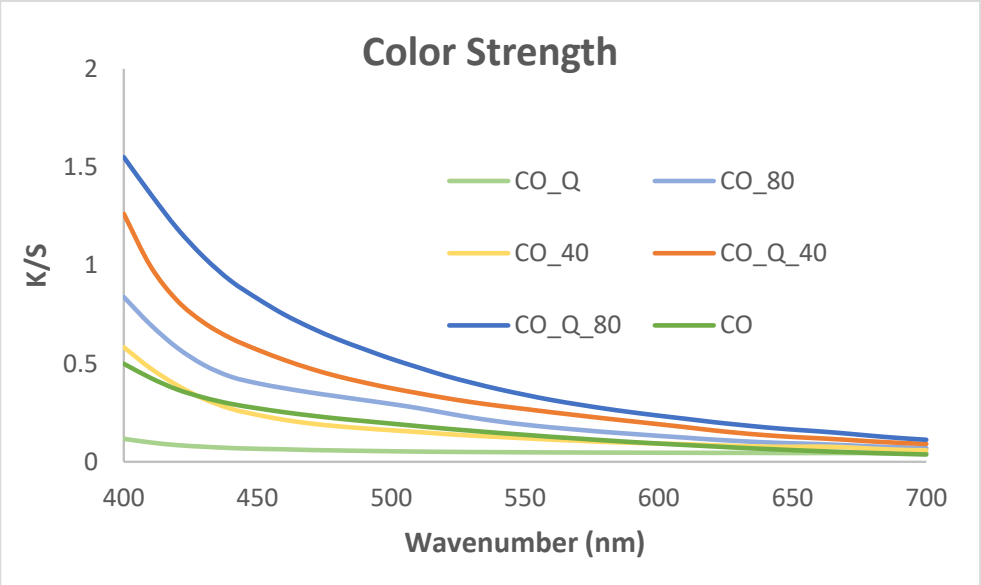

Figure 4. Colour strength. CO: cotton without dyeing; CO_Q: CO: cotton mordanted with chitosan without dyeing; CO_40: sample dyed at 40 mL/200 mL; CO_Q_40: sample mordanted with chitosan and dyed at 40 mL/200 mL; CO_80: sample dyed at 80 mL/200 mL; CO_Q_80: sample mordanted with chitosan and dyed at 80 mL/200 mL.

3.3. Colour Fastness

Once the fabrics were laundered, the evaluation of colour was measured in accordance with the standard instructions. The grey scale for degradation attributes 5 for no degradation and 1 for complete the migration of colour, and the scale for discharge means 5 no discharge and 1 completely coloured. Table 3 shows the values in grey scale for both degradation and discharge. The differences in behaviour can be observed when the concentration of the extract was increased from 40 mL/200 mL to 80 mL/200 mL. Furthermore, some variations occur when chitosan is used as a mordanting agent.

Table 3. Colour fastness. CO: cotton without dyeing; CO_Q: CO: cotton mordanted with chitosan without dyeing; CO_40: sample dyed at 40 mL/200 mL; CO_Q_40: sample mordanted with chitosan and dyed at 40 mL/200 mL; CO_80: sample dyed at 80 mL/200 mL; CO_Q_80: sample mordanted with chitosan and dyed at 80 mL/200 mL.

Reference	Discharge		Degradation
	Co	Wo	
CO	5	5	5
CO_ Q	5	5	5
CO_40	4	4/5	3
CO_40_Q	4/5	5	4/5
CO_80	4	4/5	2/3
CO_80_Q	4/5	5	4/5

3.4. Ultraviolet Protection Factor (UPF)

The UPF has been calculated for every sample dyed at different concentrations (40 mL/200 mL and 80 mL/200 mL) and for the ones no mordanted or mordanted with quitosan. Furthermore, the samples treated according to ISO 105:C06 to evaluate colour fastness were also subjected to the UPF test. Results are shown in Table 4.

Table 4. UPF from cotton samples.

Sample	Dyed		Dyed + Washed	
	UPF 40 mL	UPF 80 mL	UPF 40 mL	UPF 80 mL
Cotton	1.31	1.31	1.45	1.45
Cotton + chitosan	1.37	1.37	1.51	1.51
Cotton + red tea	8.01	9.25	6.23	7.89
Cotton + chitosan + red tea	16.7	37.48	16.23	35.98

Cotton or the mordanted one has no ultraviolet protection, as can be observed in Table 4. The tea extract increased the UPF up to values around 8 for dyes with 40 mL/200 mL of extract and around 10 for samples treated with 80 mL/200 mL; however, the chitosan proved his action by increasing the UPF for the dyed samples and not for the white ones. After colouring the chitosan-padded cotton, the UPF value went up to 16.7 for dyes with 40 mL of extract and around 37.5 for samples treated with 80 mL. The value increased considerably when using the chitosan, when compared to the cotton dyed without treatment. The additional hydroxyl groups from chitosan helped to increase the H-bonding between the polyphenols and the cotton fibres. As can be observed from Table 4, the samples without tea extract treatment (cotton and cotton + chitosan) showed a slight increase in UPF. However, a considerable decrease was observed for dyed samples, gap which was higher for non-mordanted samples.

The AS/NZ 4399: 1996 standard [43] was used to classify the textiles according to the UPF range. Table 5 shows that cotton mordanted with chitosan and dyed with 40 mL of extract, as its UPF was around 16, only allowed for 6.7% of the radiation to pass through, whereas the sample dyed with 80 mL of extract shifted to 37.5, which means that the UVR transmission is lower than 4%, showing very good protection.

Table 5. UPF classification.

UPF Range	Protection Category	Effective UVR Transmission (%)	Rating
15–24	Good	6.7–4.2	15–20
25–39	Very good	4.1–2.6	25, 30, 35
50–50, 50+	Excellent	≤2.5	40, 45, 50, 50+

4. Discussion

Some authors [44] demonstrated the possibility of dyeing cotton or other cellulosic fibres with tea and analysed the influence of mordant on the dye uptake. It has also been demonstrated previously that chitosan can be suitable for dyeing tea onto cotton fibres [38,39]. In this study, we demonstrated that the colour can be enhanced by the use of chitosan as a mordant, but it is not necessary to dye at around 100 °C, as commonly studied [44–46]. Dyeing at 50 °C is feasible and enables to avoid degradation of some polyphenols [33] and, from the sustainability point of view, save time and energy.

The results show that, despite the concentration of dye, polyphenols move towards the fibre faster than tannins. It is necessary to keep the treatment for 45 min until the condensed tannins considerably increase its presence in mordanted cotton, whereas 15 min is enough to absorb around the 15% of polyphenols in cotton, or 30 min if the cotton is the mordanted one. This means that further studies can be conducted in order to determine if 45 min is enough to allow the phytoconstituents from tea to completely migrate towards the fibre. What can be clearly stated is the fact that chitosan is a suitable mordant for red tea dyeing. Some references evidence the possibility of incorporating polyphenols from tea into the chitosan films [22,47]. Some previous works show the possibility of including tannins with chitosan in some composites [48], and there is no evidence in previous studies regarding the behavior when dyeing. Samples treated with chitosan show higher values of tannins, specially condensed ones, and slight differences for polyphenols. Apparently, it seems that chitosan shows a higher affinity towards condensed tannins than towards hydrolysable tannins or polyphenols in general. Tannins concentration on the cellulosic fibres treated with chitosan doubles the one from cotton fabrics. According to some literature [25], as it has been stated before, the reaction with dyes depends on the nature of the dyeing process. When the pH is alkaline, the hydroxyl groups are the receptors, whilst in acidic conditions, the amino groups are the ones that will react with the dye. Consequently, it is due to the conditions of the bath (pH = 6.3) with condensed tannins that can react easily to the rest of the phytoconstituents [49]. If no mordant is present, it is supposed to be a hydrogen bonding reaction between hydroxyl from condensed tannins and hydroxyl groups from cellulose. The presence of chitosan as a mordant under slightly acidic conditions enhances a reaction between the hydroxyl groups from condensed tannins and the amino groups from chitosan [50]. These findings enhance our understanding of how phytocomponents react with cotton or chitosan.

Regarding the colour fastness, it can be noticed that there is worst behaviour for samples treated at higher concentrations of the extract, something which could be expected due to the increase in concentration for the colouring agent. Additionally, the mordanting treatment improves the results, dealing a considerable improvement to the discharge and degradation, as well. This is evidence of some kind of covalent bonding, and not an ionic one, such as the one shown in Figure 1, occurs between the cotton fibre, the chitosan, and obviously, the phytocomponents.

Fabric dyed with chitosan shows double protection against UV (UPF), compared to non-mordanted fabrics. There is a clear difference in the UPF behaviour between the mordanted and non-mordanted fabrics. Considering that the content in polyphenols is similar for every sample, the tannins content is higher for the mordanted fabrics, and similar behaviour is appreciated for tannins, we can affirm that tannins must be responsible of the UPF protection conferred to the cellulosic fibres. Further research in this field to analyse other polyphenols, such as flavonoids, catechin, etc., needs to be performed. When samples are laundered, the increase on non-dyed samples can be attributed to a slight shrinkage in the fabric, which narrows the fabric's pore, preventing the radiation from passing through them. Dyed samples show a decrease in the ultraviolet protection. This variation is higher for non-mordanted samples, in comparison to mordanted ones, which corroborated reactions between phytocomponents and chitosan.

Despite polyphenols migrating towards the fibre considerably fast, this speed is not causing an irregular dyed surface, as could be observed from the surface analysis and by

the colourimetric measurement. What is evident is the increase in colour from the sample bio-mordanted with chitosan. This allows us to confirm the effectiveness of chitosan as a mordant. The colour difference ΔE*ab between the chitosan-padded fabric and chitosan-padded fabric coloured with red tea extracts is far greater than the cotton fibres coloured with the extracts without the bio-mordant process, as could be expected [45,46], due to the use of a mordant. This can lead to the following conclusion: the chitosan can uptake more phytocomponents, which can lead to a bigger colour difference. This difference between mordanted samples can be due to the fact that the chitosan cross-links with cotton, deriving higher -OH groups available on the fibre surface and enhancing the active sites capable of reacting between the phytocomponents and the chitosan. This is corroborated by the lower reduction in UPF from mordanted samples once they have been laundered, in comparison to non-mordanted ones.

Cotton dyed with 80 mL/200 mL(CO_80) shows similar colour to the one mordanted and dyed with 40 mL/200 mL(CO_Q_40) ΔE*ab is approximately 3.5, showing CO_80 less colour intensity than CO_Q_40. This means that the chitosan mordanted samples can show a similar intensity in coulour to a double concentration of extract without mordanting. It is worth noting that, when the fabric is dyed, it barely increases by 8 points in the UPF; however, samples with quitosan show low differences in colour (ΔE*ab is approximately 3.5), but the difference in UPF is considerably different (9.25 for CO_80 and 16.7 for CO_Q_40). This can lead to thinking that the chitosan can uptake more phytocomponents, which implies not a high colour difference, but a considerable increase in UPF. Thus, as a conclusion, we can affirm that there are some phytocomponents that do not confer colour to the samples, but increase other properties, such as the ultraviolet protection. Results from colour strength (K/S), as expected, are in accordance with the colour differences reported in Table 2.

This increase in colour has also been reflected by the increase in the ultraviolet protection reaching levels of protection that can be considered good. More research can be conducted with different concentrations of tea leaves or different kinds of tea (green, black, white), or even other plants, in order to optimise the process and fulfill the goal of reaching excellent protection.

5. Conclusions

Some phytocomponents are responsible for functionalizing cotton with properties such as ultraviolet protection. As was expected, chitosan can be used as a bio-mordant to dye cotton with red tea. In this study, we have demonstrated that there are some phytoconstituents in tea, such as condensed tannins, which react with chitosan faster than the hydrolysable ones. We demonstrated that it is important to increase their presence in cellulosic fibres by means of pre-mordanting the fibre.

One of the more significant findings to emerge from this study is that chitosan enhances the UPF protection higher than the colour intensity, so we can conclude that chitosan acts not only with coloured components, but also with active and non-coloured ones, as well. Chitosan reaction offers good values for colour fastness, which suggests that there is a strong linkage with the extract compounds. Another finding from the experiments is the fact that the phytocomponents that confer UPF protection are tannins.

Author Contributions: Conceptualization, L.C. and E.B.-B.; methodology, J.G.-P.; formal analysis, J.G.-P. and L.C.; investigation, L.L.; data curation, J.G.-P.; writing—original draft preparation, I.M. and J.G.-P.; writing—review and editing, L.C.; visualization, I.M. and J.G.-P.; supervision, P.D.-G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Spanish Ministry of Science, Innovation, and Universities, under the Retos de la investigación, I+D+I orientada a los retos de la sociedad program on the 2018 call because of the project reference RTI2018-094014-B-I0.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kadolph, S. Natural dyes: A traditional craft experiencing new attention. *Delta Kappa Gamma Bull.* **2008**, *75*, 14–17.
2. Phan, K.; Van Den Broeck, E.; Speybroeck, V.; De Clerck, K.; Raes, K.; De Meester, S. The potential of anthocyanins from blueberries as a natural dye for cotton: A combined experimental and theoretical study. *Dye. Pigment.* **2020**, *176*, 108180. [CrossRef]
3. Saxena, S.; Raja, A. Natural dyes: Sources, chemistry, application and sustainability issues. In *Roadmap to Sustainable Textiles and Clothing*; Springer: Berlin/Heidelberg, Germany, 2014; pp. 37–80.
4. Shahid, M.; Mohammad, F. Recent advancements in natural dye applications: A review. *J. Clean. Prod.* **2013**, *53*, 310–331. [CrossRef]
5. Vadood, M.; Haji, A. Application of ANN Weighted by Optimization Algorithms to Predict the Color Coordinates of Cellulosic Fabric in Dyeing with Binary Mix of Natural Dyes. *Coatings* **2022**, *12*, 1519. [CrossRef]
6. Mohammad, F.; Shahid, I. Natural colorants in the presence of anchors so-called mordants as promising coloring and antimicrobial agents for textile materials. *ACS Sustain. Chem. Eng.* **2015**, *3*, 2361–2375.
7. Haji, A.; Nasiriboroumand, M.; Qavamnia, S.S. Cotton dyeing and antibacterial finishing using agricultural waste by an eco-friendly process optimized by response surface methodology. *Fibers Polym.* **2018**, *19*, 2359–2364. [CrossRef]
8. Toprak-Cavdur, T.; Uysal, S.; Gisbert-Paya, J. Dyeing Recycled Cotton Fibers Using Curcuma Longa and Pterocarpus Santalinus Natural Dyes and Bio-mordant Chitosan. *J. Nat. Fibers* **2022**, *19*, 13736–13752. [CrossRef]
9. Bou-Belda, E.; Indrie, L.; Ilies, D.C.; Hodor, N.; Berdenov, Z.; Herman, G.; Caciora, T. Chitosan–a non-invasive approach for the preservation of historical textiles. *Ind. Text.* **2020**, *71*, 576–579. [CrossRef]
10. Buyukakinci, Y.B.; Guzel, E.T.; Karadag, R. Organic cotton fabric dyed with dyer's oak and barberry dye by microwave irradiation and conventional methods. *Ind. Text.* **2021**, *72*, 30–38. [CrossRef]
11. Houshyar, S.; Amirshahi, H.S. Treatment of Cotton with Chitosan and Its Effect on Dyeability with Reactive Dyes. *Iran. Polym. J.* **2002**, *11*, 259–301.
12. Yadav, M.; Singh, N.; Kaur, A.; Sahu, O. Antibacterial activity assessment of woolen fabric treated with natural dyes and chitosan. *Agric. Nat. Resour.* **2019**, *53*, 188–196.
13. Butola, B.S. Effect of chitosan biological macromolecule on colorimetric analysis and radical scavenging activity of linen using pineapple peel extract biomolecules. *Int. J. Biol. Macromol.* **2019**, *124*, 708–715.
14. Saini, S.; Gupta, A.; Singh, N.; Sheikh, J. Functionalization of linen fabric using layer by layer treatment with chitosan and green tea extract. *J. Ind. Eng. Chem.* **2020**, *82*, 138–143. [CrossRef]
15. Haji, A. Dyeing of cotton fabric with natural dyes improved by mordants and plasma treatment. *Prog. Color Color. Coat.* **2019**, *12*, 191–201.
16. Kampeerapappun, P.; Phattararittigul, T.; Jittrong, S.; Kullachod, D. Effect of chitosan and mordants on dyeability of cotton fabrics with Ruellia tuberosa Linn. *Chiang Mai J. Sci.* **2010**, *38*, 95–104.
17. Hong, S.J.; Jeon, D.W.; Kim, J.J.; Choi, I.R. Effect of chitosan and mordant treatments on the color change of cotton and nylon fabrics dyed using rhusjara ica. *Res. J. Costume Cult.* **2005**, *13*, 380–390.
18. Uzun, I. Kinetics of the adsorption of reactive dyes by chitosan. *Dye. Pigment.* **2006**, *70*, 76–83. [CrossRef]
19. Sakkayawong, N.; Thiravetyan, P.; Nakbanpote, W. Adsorption mechanism of synthetic reactive dye wastewater by chitosan. *J. Colloid Interface Sci.* **2005**, *286*, 36–42. [CrossRef]
20. Mahmoodi, N.M.; Salehi, R.; Arami, M.; Bahrami, H. Dye removal from colored textile wastewater using chitosan in binary systems. *Desalination* **2011**, *267*, 64–72. [CrossRef]
21. Sánchez-Duarte, R.G.; Sánchez-Machado, D.I.; López-Cervantes, J.; Correa-Murrieta, M.A. Adsorption of allura red dye by cross-linked chitosan from shrimp waste. *Water Sci. Technol.* **2012**, *65*, 618–623. [CrossRef]
22. Kim, S.-H. Dyeing characteristics and UV protection property of green tea dyed cotton fabrics. *Fibers Polym.* **2006**, *7*, 255–261. [CrossRef]
23. Bhuiyan, R.M.; Shaid, A.; Khan, M.A. Cationization of Cotton Fiber by Chitosan and Its Dyeing with Reactive Dye without Salt. *Chem. Mater. Eng.* **2014**, *2*, 96–100. [CrossRef]
24. Vayalil, P.K.; Mittal, A.; Hara, Y.; Elmets, C.A.; Katiyar, S.K. Green tea polyphenols prevent ultraviolet light-induced oxidative damage and matrix metalloproteinases expression in mouse skin. *J. Investig. Dermatol.* **2004**, *122*, 1480–1487. [CrossRef] [PubMed]
25. Shin, Y.; Yoo, D.I. Use of chitosan to improve dyeability of DP-finished cotton (II). *J. Appl. Polym. Sci.* **1998**, *67*, 1515–1521. [CrossRef]
26. Rippon, J.A. Improving the dye coverage of immature cotton fibres by treatment with chitosan. *J. Soc. Dye. Colour.* **1984**, *100*, 298–303. [CrossRef]
27. Elmets, C.A.; Singh, D.; Tubesing, K.; Matsui, M.; Katiyar, S.; Mukhtar, H. Cutaneous photoprotection from ultraviolet injury by green tea polyphenols. *J. Am. Acad. Dermatol.* **2001**, *44*, 425–432. [CrossRef]
28. Liu, X.D.; Nishi, N.; Tokura, S.; Sakairi, N. Chitosan coated cotton fiber: Preparation and physical properties. *Carbohydr. Polym.* **2001**, *44*, 233–238. [CrossRef]
29. Xiong, X.; Sun, J.; Hu, D.; Xiao, C.; Wang, J.; Zhuo, Q.; Dai, L. Fabrication of polyvinyl alcohol hydrogels with excellent shape memory and ultraviolet-shielding behavior via the introduction of tea polyphenols. *RSC Adv.* **2020**, *10*, 35226–35234. [CrossRef]
30. Bonet-Aracil, M.A.; Belda, B.E.; Diaz, P.; Sebastia, N. UV protection from cotton fabrics dyed with different tea extracts. *Dye. Pigment.* **2016**, *134*, 448–452. [CrossRef]

31. Kanwar, J.; Taskeen, M.; Mohammad, I.; Huo, C.; Chan, T.H.; Dou, Q.P. Recent advances on tea polyphenols. *Front. Biosci. (Elite Ed.)* **2012**, *4*, 111–131. [CrossRef]
32. Naima, R.; Oumam, M.; Hannache, H.; Sesbou, A.; Charrier, B.; Pizzi, A.; Charrier–El Bouhtoury, F. Comparison of the impact of different extraction methods on polyphenols yields and tannins extracted from Moroccan Acacia mollissima barks. *Ind. Crops Prod.* **2015**, *70*, 245–252. [CrossRef]
33. Gironi, F.; Piemonte, V. Temperature and solvent effects on polyphenol extraction process from chestnut tree wood. *Chem. Eng. Res. Des.* **2011**, *89*, 857–862. [CrossRef]
34. Lambrecht, L.; Gisbert-Payá, J.; Bou-Belda, E.; Bonet, M.Á. Optimization of tea extracts composition to dye cotton. Time and temperature influence. *J. Appl. Res. Technol. Eng.* **2020**, *1*, 3–7. [CrossRef]
35. Hebeish, A.; El-Naggar, M.E.; Fouda, M.M.G.; Ramadan, M.A.; Al-Deyab, S.S.; El-Rafie, M.H. Highly effective antibacterial textiles containing green synthesized silver nanoparticles. *Carbohydr.-Polym.* **2015**, *86*, 936–940. [CrossRef]
36. Sheshir, M.H. *Textile Chemical Dictionarey*; Wet Processing Technology: Allentown, PA, USA, 2014; p. 66.
37. Singleton, V.; Rossi, J. Colorimetry of total phenolics with phospomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.
38. Bossu, C.F. Flow injection system for hydrolysable tannin determination. *Microchem. J.* **2006**, *84*, 88–92. [CrossRef]
39. Schofield, P.; Mbugua, D.M.; Pell, A.N. Analysis of condensed tannins: A review. *Anim. Feed. Sci. Technol.* **2001**, *91*, 21–40. [CrossRef]
40. Scalbert, A.; Monties, B.; Janin, G. Tannins in wood: Comparative study on the phenolic acids identified and quantified in dry beans using HPLC as affected by different extraction and hydrolysis methods. *Food Chem.* **1989**, *113*, 1324–1329. [CrossRef]
41. *ISO 105-C06:2010*; Colour Fastness to Commercial and Domestic Laundering. ISO: Geneva, Switzerland, 2010.
42. Gabrijelčič, H.; Urbas, R.; Sluga, F.; Dimitrovski, K. Influence of fabric constructional parameters and thread colour on UV radiation protection. *Fibres Text. East. Eur.* **2009**, *17*, 46–54.
43. *AS/NZ 4399:1996*; Sun Protecting Clothing. Evaluation and Characterization. Standards New Zealand: Wellington, New Zealand, 1996.
44. Dawson, T.L.; Todd, J.C. Dye Diffusion-The Key to Efficient Coloration. *J. Soc. Dye. Colour.* **1979**, *95*, 417–426. [CrossRef]
45. Deo, H.T.; Desai, B.K. Dyeing of cotton and jute with tea as a natural dye. *Color. Technol.* **1999**, *115*, 224–227. [CrossRef]
46. Campos, J.; Diaz-Garcia, P.; Montava, I.; Bonet-Aracil, M.; Bou-Belda, E. Chitosan pretreatment for cotton dyeing with black tea. In *IOP Conference Series-Materials Science and Engineering*; IOP Publishing: Bristol, UK, 2017; p. 254.
47. Mansour, R.; Ben Ali, H. Investigating the Use of Chitosan: Toward Improving the Dyeability of Cotton Fabrics Dyed with Roselle (*Hibiscus sabdariffa* L.). *J. Nat. Fibers* **2019**, *18*, 1007–1016. [CrossRef]
48. Wang, L.; Dong, Y.; Men, H.; Tong, J.; Zhou, J. Preparation and characterization of active films based on chitosan incorporated tea polyphenols. *Food Hydrocoll.* **2013**, *32*, 35–41. [CrossRef]
49. Gao, H.X.; He, Z.; Sun, Q.; He, Q.; Zeng, W.C. A functional polysaccharide film forming by pectin, chitosan, and tea polyphenols. *Carbohydr. Polym.* **2019**, *215*, 1–7. [CrossRef] [PubMed]
50. Zhu, X.; Hou, X.; Ma, B.; Xu, H.; Yang, Y. Chitosan/gallnut tannins composite fiber with improved tensile, antibacterial and fluorescence properties. *Carbohydr. Polym.* **2019**, *226*, 115311. [CrossRef]