Carbohydrate Polymers 77 (2009) 536–543

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Cross-linking chitosan into UV-irradiated cellulose fibers for the preparation of antimicrobial-finished textiles

Diana Alonso [a], Miquel Gimeno [b], Roberto Olayo [a], Humberto Vázquez-Torres [a], José D. Sepúlveda-Sánchez [a], Keiko Shirai [a,*]

[a] Universidad Autónoma Metropolitana. Av. San Rafael Atlixco No. 186, Col. Vicentina, 09340 Mexico City, Mexico
[b] Facultad de Química, Departamento Alimentos y Biotecnología, Universidad Nacional Autónoma de México, Ciudad Universitaria, 04510 Mexico City, Mexico

ARTICLE INFO

Article history:
Received 13 May 2008
Received in revised form 19 January 2009
Accepted 26 January 2009
Available online 6 February 2009

Keywords:
Cellulose fiber
Chitin
Chitosan
Cross-linking
Antimicrobial-finished textile
UV-irradiation

ABSTRACT

Chitosan cross-linked cellulose fibers were prepared using non-toxic procedures in order to confer antimicrobial properties to cellulose fibers. Citric acid was used as the cross-linker and NaH_2PO_4 as catalyst in previously UV-irradiated cellulose fibers. Further heat dried-cure process and washing with detergent, water and acetic acid (0.1 M) gave a maximum incorporation of chitosan of 27 mg per gram of functionalized textile. The thermogravimetric analysis of the material with the highest chitosan content showed an increased thermal stability compared to cellulose and chitosan. The UV-irradiation induced morphological changes, such as less entangled cellulose fibers, as observed by scanning electron microscopy, which was prompted to enhance the chitosan incorporation. The biomass and spore germination percentage of *Penicillium chrysogenum* and colony forming units per millilitre for *Escherichia coli* decreased significantly on the composed materials as compared to raw cellulose fiber and it was similar to that obtained with a commercial antimicrobial cellulose fiber.

1. Introduction

Bacteria, gram positive and gram negative, as well as fungi have been commonly found in textiles, including bedclothes and clothing. Many of these microorganisms are pathogens or opportunistic pathogens quite often related to nosocomial infections (Takai et al., 2002). *Escherichia coli* and *Penicillium chrysogenum* are very common pollutants that cause diseases and deteriorate textiles by discoloration, disintegration as well as emission of foul smells. These microorganisms are ubiquitous, for instance, *P. chrysogenum* is an airborne contaminant on heating, ventilating and air conditioning systems and it is pointed that might cause health problems such as rhinitis or asthma (Chang, Foarde, & VanOsdell, 1996). Therefore, the development of non-toxic synthetic process for the preparation of biodegradable and biocompatible textiles with antimicrobial capacities, also known as antimicrobial-finished textile products is encouraged. These antimicrobial-finished textile products usually contain inhibitory compounds such as silver, quaternary ammonium chloride, metals, aromatic, halogen compounds and chitosan (Kenawy, Worley, & Broughton, 2007; Takai et al., 2002).

Chitosan, the deacetylated form of the polysaccharide chitin, which is mainly found in the exo-skeleton of crustaceans, is the second most abundant polysaccharide in nature which has received much attention due to its wide versatility, non-toxicity and antimicrobial properties. Its antimicrobial property is related to the interaction of charges between the amino groups of the polycationic form of chitosan with the microbial cell walls (Fang, Chan, Mao, & Leong, 2001). The degradation of proteins and other intracellular constituents then occur, as well as alterations on the permeability of the microbial cell, which induces the loss of essential nutrients and eventual death (Chan, Mao, & Leong, 2001). It has been shown that chitosan displays fungistatic effect on *Aspergillus niger* due to the direct interaction with the cell wall of the spores (Plascencia, Viniegra, Olayo, Castillo, & Shirai, 2003), therefore it induces delay in the spore germination. Due to this antimicrobial activity, chitosan has been applied in several applications such as the control of common postharvest fungal phytopathogens such as *Botrytis cinerea, Rhizopus stolonifer, Penicillium digitatum* (Pacheco et al., 2008; Tharanathan & Kittur, 2003). Chitosan derivatives have been prepared by several methods in order to increase the antimicrobial activity by means of grafting with *N*-alkyl groups, quaternary ammonium groups, esters among others (Kenawy et al., 2007). In this sense, the chemical fixation of the chitosan by covalent cross-linking into the cellulose or wool matrices may improve the endurance and efficiency of the resultant functionalised textile (Lim & Hudson, 2004; Zhang, Chen, Ji, Huang, & Chen, 2003). The chemical fixation treatment applied to cellulose fibers is known as durable press finishing (DP) and it is of increasing

* Corresponding author. Tel.: +52 5 5804 49 21; fax: +52 5 5804 47 12.
E-mail address: smk@xanum.uam.mx (K. Shirai).

0144-8617/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2009.01.027

interest in textile industries as to increase textile properties, such as anti-odour, low-shrinkage capacities and wrinkle resistance, as well as antimicrobial properties. DP involves nowadays the use of cross-linking agents, which are mainly composed of formaldehyde reactants. These materials have several constraints like the reduction of mechanical properties, fiber degradation, but above all, the release of toxic and irritant formaldehyde vapours during DP process and even during storage (Yang, 1993a, 1993b). In order to address this issue, polycarboxylic acids, such as butanetetracarboxylic acid and citric acid (CA), have been used as alternatives to formaldehyde reactants. However, earlier reported procedures to attain the covalent linkage of the chitosan into textiles often involved the use of toxic reagents or additives, such as glutaric dialdehyde or other cross-linkers (Schiffman & Schauer, 2007; Zhang et al., 2003). Instead, the use of CA and low toxic oxidizing agents, such as potassium permanganate and sodium hypophosphite, have been shown to promote an effective cross-linking between chitosan and the cellulose by ester formation (Chung, Lee, & Kim, 1998; Lim & Hudson, 2004). Although the production of such materials still involved some risks associated with the human health and environment due to the use of additives in their manufacture.

The UV-irradiation has been proposed as a suitable procedure to achieve adequate polymer surface modifications by means of the generation of reactive sites, which can be of further use to attach many compounds of interest (Goddard & Hotchkiss, 2007). Exposure of polysaccharides to UV-irradiation is reported to enhance chemical reaction due to photooxidative degradation. This photodegradation process involves photochemical reactions, such as chain scission, dehydroxylation and dehydrogenation, which are responsible for the formation of free radicals on nitrogen or oxygen atoms of the polysaccharide (Goddard & Hotchkiss, 2007; Rabek, 1996; Schiffman & Schauer, 2007).

In the present work, pre-UV-irradiated raw cellulose fibers were treated with acidic aqueous solutions of chitosan prepared with different concentrations of CA as cross-linker, as well as several ratio of sodium phosphate as reaction catalyst. The conditions were evaluated in order to attain the highest covalent attachment of chitosan using non-toxic methodologies and reagents (Scheme 1). All the synthesized fibers and a commercial one, which contained quaternary ammonium salts as chemical antimicrobial compounds, were evaluated on the inhibition of growth of *P. chrysogenum* and *E. coli*.

2. Experimental

2.1. Materials

Citric acid monohydrate ($C_6H_8O_7{\cdot}H_2O$) and sodium phosphate (NaH_2PO_4) were purchased from J.T. Baker (Mexico) and used as supplied. Chitosan was provided by Heppe Gmb (Germany) and used as supplied. The fraction of acetylated units (F_A) was 0.1886 determined by ^{1}H NMR spectroscopy and the average molecular weight was 387900 g/mol determined by Mark–Houwink–Sakurada equation using intrinsic viscosities (Pacheco et al., 2008). Raw cellulose fiber was a kind gift from Casa Miyako (Mexico). Commercial cellulose fiber with ammonium salts as antimicrobial agent was supplied by Comercializadora del Rio (Mexico).

2.2. Microorganisms

Freeze dried *P. chrysogenum* (CECT 2267 from Spanish Culture Type Collection) was kept at −20 °C and it was subsequently activated in dextrose Sabouraud broth (Bioxon, USA), then it was cultivated at 30 °C during 4 days. *P. chrysogenum* was cultivated in potato dextrose agar, PDA, (Bioxon, USA) and maintained at 4 °C until used.

Similar procedure was carried out for *E. coli* (NRRLB 14218 from USDA ARS Culture Collection). Bacteria was cultivated and maintained at 4 °C in nutrient agar (Bioxon, USA) until used.

2.3. Fungal spore and bacterial cell suspensions as inoculum

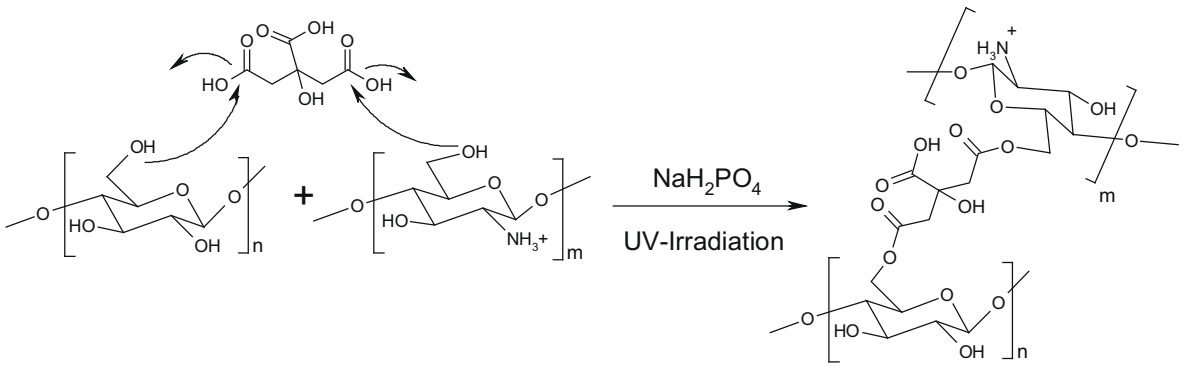
P. chrysogenum was cultivated in Czapeck agar media with the following composition (g/L): sucrose (30), $NaNO_3$ (1), $MgSO_4{\cdot}7H_2O$ (0.5), KCl (0.5), $K_2PO_4{\cdot}3H_2O$ (1) and $FeSO_4{\cdot}7H_2O$ (0.01). The spore suspension was obtained by agitation of these fungal cultures in a Tween-80 solution at 0.1 vol/vol%. The spores were counted in a Neubauer chamber using a light microscope (Axiostar-plus Zeiss, Germany) and they were adjusted to a final concentration of 1×10^5 spores/mL.

Bacteria were inoculated in 50 mL of nutrient broth and incubated at 37 °C for 24 h. Then, several decimal dilutions were prepared in NaCl solution (0.09 wt/vol%) and inoculated in nutrient agar plates. Cell suspensions were used as inoculum for further experimentation with 1×10^7 CFU/mL.

2.4. Cellulose fiber treatment procedures

2.4.1. Cellulose cross-linked chitosan without NaH_2PO_4 catalyst addition

The acetic acid aqueous solution was evaluated as suitable acidifier for the chitosan cross-linking reaction with cellulose. Raw cellulose fibers were immersed into chitosan aqueous solution 1.5 wt/vol% in acetic acid (0.1 M) with pH 3. Then, different amounts of citric acid, up to concentrations in final solutions of 4 wt/vol%, 8 wt/vol% and 12 wt/vol%, were added to the mixture and heated at 70 °C for 5 min. Cellulose fiber was removed from the solution and cured in an oven at 130 °C for 3 min. The samples were dried



Scheme 1. Proposed scheme reaction for cross-linking chitosan to cellulose using citric acid, NaH_2PO_4 and UV-irradiation.

under vacuum at room temperature, accurately washed with commercial ionic soap and rinsed with distilled water. The samples were further washed with acetic acid solution (0.1 M) in order to remove the excess of the non-reacted chitosan. Then, they were rinsed with distilled water, dried overnight under vacuum and weighted prior to analyses. This washing–drying procedure was repeated until the weight of the fiber was invariable.

2.4.2. Cellulose cross-linked chitosan with NaH_2PO_4 catalyst addition

Improved materials, with higher chitosan content, were prepared in a modified procedure based on the addition of 2.3 wt/vol% of NaH_2PO_4 before the thermal pre-treatment and the absence of acetic acid. Citric acid was used as the only acidifier as well as cross-linker agent and it was added to the aqueous solutions at three different concentrations; 4 wt/vol%, 8 wt/vol% and 12 wt/vol%. The final pH of the CA–NaH_2PO_4–chitosan solutions was 3.4, 2.83 and 2.4 for 4 wt/vol%, 8 wt/vol% and 12 wt/vol% of CA, respectively. The effect of the NaH_2PO_4 concentration was also studied in 4 wt/vol% citric acid aqueous solution with two different catalyst concentrations of 9.2 wt/vol% and 18.4 wt/vol% at the thermal pre-treatment at 70 °C. The pH of the CA–NaH_2PO_4–Chitosan solutions was 3.8 and 4.1 for 9.2 wt/vol% and 18.4 wt/vol% of NaH_2PO_4, respectively.

2.4.3. Cellulose cross-linked chitosan with NaH_2PO_4 catalyst addition and previous UV-irradiation of the raw cellulose samples

A batch of synthesized materials were prepared by previous UV-irradiation of the raw cellulose fibers with a high intensity UV lamp (UVGL-58, Ultra-Violet Products, USA) at wavelength of 254 nm with different times of exposure, 4 h, 8 h and 20 h and followed by identical procedure described above using CA concentrations of 4 wt/vol%, 8 wt/vol% and 12 wt/vol% with a fixed NaH_2PO_4 concentration of 2.3 wt/vol%.

2.5. Determination of fungal growth and spore germination rates in culture media within the obtained materials

Spore suspension was added to 40 mL of Czapeck broth in 125 mL flask until a concentration of 1×10^5 spores/mL. Commercial cellulose fiber, raw cellulose fiber and the different materials produced herein were cut in 2 cm^2 squares and added into the flasks (Table 1). Blank samples were also prepared by inoculation of the media without any cellulose fiber. The inoculated flasks were incubated at 25 °C with mechanical agitation at 200 rpm. Samples (flasks) were taken by duplicates every 6 h and up to a final time of 120 h. The content of each flask was filtered through a Whatman No. 40 paper filter and the biomass weight was gravimetrically determined.

Spore germination assays were carried out in 125 mL flasks with 40 mL of Czapeck broth equipped with 48 glass coverslips. Then, they were inoculated until final concentration of 1×10^5 spores/mL. Samples were taken every 2 h by duplicates and up to a final time of 24 h. 200 spores per glass coverslip were counted using a light microscope (Axiostar-plus Zeiss, Germany) after fixation with a formaldehyde solution at 3.7 wt/vol%, Triton X-100 0.2 wt/vol% and potassium phosphate buffer 50 mM (pH 7). A given spore was considered germinated when the length of its germinal tube reached one-half of the spore diameters following a similar methodology reported by Plascencia et al. (2003).

Fungal biomass and germinated spore percentages were adjusted with logistic model (Eq. (1)) using software Origin 7.0 SR0 (Microsoft):

$$Y(t) = \frac{a}{(1 + e^{(b-ct)})} \quad (1)$$

where Y is biomass (X_t in mg/mL) or germinated spores (S_t in%) at a certain time, a is the initial value (X_0 in mg/mL, or S_0 in%), b is the maximum biomass production obtained when $\frac{dy}{dt} = 0$ (X_{max} in mg/mL) or the maximum percentage of germinated spores (S_{max} in%) and c is the specific growth rate (μ_{max} in h^{-1}) for biomass when $X << X_{max}$, or the specific spore germination rate (k in h^{-1}) when $S << S_{max}$.

2.6. Determination of bacterial inhibition with the obtained materials

Evaluation of bacterial growth was carried out by inoculation of nutrient broth with cell suspension of *E. coli* in 7 mL tubes until a final concentration of 1×10^7 CFU/mL. Subsequently, 0.02 g of the commercial fiber, raw cellulose fiber without treatment and the prepared materials were added and incubated at 37 °C with stirring at 100 rpm. Samples were taken by duplicates every 4 h and up to a final time of 43 h. Each sample was decimally diluted in saline solution 0.09 wt/vol% and was inoculated in nutrient agar plates. Then they were incubated at 37 °C for 24 h in order to determine viable bacterial counts (CFU/mL). The bacterial growth experimental data were adjusted to exponential model (Eq. (2)):

$$y(t) = ae^{(bt)} \quad (2)$$

where y is the cells number (N_t in CFU/mL) at certain time, a is the initial value (N_0 in CFU/mL), b is the growth rate (μ in h^{-1}). The percentage of inhibition (%I) was calculated considering the cell number in the blank (inoculated culture media without any fiber) and compared to each material.

2.7. Materials characterization

The elemental analyses on nitrogen content incorporation to the treated fibers were determined in a CHN Perkin-Elmer 2400 series (Connecticut, USA). A modification of the technique with ninhydrin reported by Chung et al. (1998) was also used to determine the concentration of nitrogen, as free amino group, from the chitosan in the prepared cellulose fibers. The obtained materials were pulverized until a particle size of 1 mm. Then, 1 mL of distilled water was added to 0.05 g of treated sample and 1 mL of ninhydrin solution at pH 5.8 (Beckman Coulter, USA). The mixture was boiled in water for 10 min and paper filtered through a Whatman No. 40 in a similar procedure as that reported by Sun, Lin, Weng, and Chen (2006). The absorbance was spectrophotometrically

Table 1
Composition of several formulations for the functionalization of the cotton fibers with chitosan.

Entry[a]	Acetic acid[b] (M)	Citric acid (wt/vol%)	NaH_2PO_4 (wt/vol%)	Time of UV-irradiation[c] (h)
1	0.1	4	0	0
2	0.1	8	0	0
3	0.1	12	0	0
4	0	4	0	0
5	0	4	2.3	0
6	0	8	2.3	0
7	0	12	2.3	0
8	0	4	9.2	0
9	0	4	18.4	0
10	0	4	2.3	4
11	0	4	2.3	8
12	0	4	2.3	20
13	0	8	2.3	8
14	0	12	2.3	8

[a] All entries were treated with chitosan at 1.5 wt/vol% and with thermal pre-treatment at 70 °C/5 min, dry curing at 130 °C/3 min.
[b] Acetic acid solution at 0.1 M as chitosan solvent.
[c] UV-irradiation of raw cotton before treatment with chitosan solution.

determined at 570 nm (Jenway 6305, UK), using chitosan as standard. All determinations were carried out by triplicates.

Polymer loading (PL) was calculated according to Eq. (3):

$$PL(\%) = \left(\frac{100(W_2 - W_1)}{W_1}\right) \quad (3)$$

where W_1 and W_2 were the weights of the materials on dry basis before and after cross-linking procedure, respectively.

Thermogravimetric analyses (TGA) were recorded on a Perkin-Elmer, Pyris 1 TGA (Connecticut, USA) with a heating rate of 10 °C/min within a range of 30–450 °C.

Infrared spectra (FTIR) were obtained in a Perkin-Elmer Spectrum GX FTIR System (Connecticut, USA) from samples of each step of the preparation procedure described in the experimental Section 2.4. Each sample was 120-times scanned against a blank of KBr pellets. Samples were coated with carbon and gold prior examination in the scanning electron microscope (JEOL JSM-5900 LV, Tokyo).

3. Results and discussion

3.1. Preparation and characterization of the antimicrobial-finished textiles of chitosan cross-linked to cellulose

The non-toxic procedures carried out in this work to attain chitosan functionalised cotton fibers are summarized in Table 1. The polymer loadings (PL) and chitosan contents in the textiles assessed by elemental analyses and that obtained by ninhydrin based method are summarized in Table 2. The difference observed between the chitosan concentration determined by the ninhydrin assays and that from the elemental analyses might be attributed to other factors involved in the reaction of chitosan with ninhydrin, such as F_A, degree of polymerization and pH (Prochazkova, Vårum, & Østgaard, 1999). The ninhydrin assay was based on the reaction of available free amino groups of the chitosan which produce purple colour compounds, diketohydrindylidene–diketohydrindamine, as it is reported elsewhere (Curotto & Aros, 1993; Prochazkova et al., 1999). The results of the assay pointed out that the esterification of chitosan with cellulose and citric acid occurs mainly on the hydroxyl group of the C6 of chitosan. This is relevant as the positively charged amino groups are responsible of most of the biological activities of chitosan related products. Therefore, the presence of free and non-reacted amino moieties would be preferred.

The methodologies involving the use of AA with CA or CA alone displayed the lowest chitosan incorporation being 6.78 mg/g, 6.93 mg/g, 7.75 mg/g and 6.45 mg/g for entries 1, 2, 3, and 4 in Table 2, respectively. The competition between the carboxyl moieties of AA and those from CA towards the alcohol groups in the polysaccharides was reported by Chung et al. (1998), which could diminish the final cross-linked chitosan and thus the consecution of non-durable textiles, however, the comparison of nitrogen content and PL in entries 1 and 4, in Table 2, were not different. The use of AA however is less handy due to its higher volatility and acidity than CA, which could damage the fiber. Therefore for further experimentation CA was used alone as acidifier and cross-linker.

The mechanism involved in the cross-linking of cellulose with polycarboxylic acids catalyzed by phosphates is reported to proceed throughout a two-step process, where the formation of a cyclic anhydride from the acid takes place in the first step. This intermediate is then able to react throughout its ring-opening with the alcohol groups of the polysaccharide forming the ester binding group (Blanchard & Graves, 2002; Yang, 1993a, 1993b). Yang (1993b) reported that the pH of a solution containing polycarboxylic acids should be kept in the 1.5–4.5 range in order to successfully crosslink the fibers, however, relatively high acidities are not convenient because it can damage the fibers. Instead, more ideal conditions are recommended using adequate catalysts in the pH range of 3.7–4.5 (Blanchard & Graves, 2002). In our experience, the addition of relatively low amount of NaH_2PO_4 (2.3 wt/wt%) in a chitosan solution with the presence of CA increased the fixation of the polysaccharide (entries 4–7 in Table 2), which might be explained by the presence of the phosphate and the partial sodium salt of the polycarboxylic acid to accelerate the esterification reaction (Blanchard & Graves, 2002). It is worth mentioning that the increase of phosphate (9.2 and 18.4 wt/wt%) at a fixed CA concentration (4%) resulted in a decrease of the chitosan content (entries 5 and 8, 9 in Table 2), which may be attributed to the neutralization of free-carboxyl groups, thus restricting the esterification with the chitosan hydroxyl moieties. These results are in agreement with a previous work reported by Coma, Sebti, Pardon, Pichavant, and Deschamps (2003) in which the increase in the NaH_2PO_4 concentration resulted in the decreasing cross-linking between hydroxylpropylmethylcellulose and CA.

In the experiments without catalyst, the reaction underwent low ester cross-linking to chitosan, since the contents were lower than those attained using the phosphate catalyst (entries 1–4 in Table 2).

On the other hand, it was expected that the increase in CA concentrations would increase the chitosan content in the textiles, however these synthesized materials presented similar chitosan contents (entries from 5 to 7 in Table 2). The amount of the catalyst could have affected the cross-linking reaction since it might be

Table 2
Chitosan content in the treated cotton fibers[a].

Entry	Treatments	PL[b] (%)	Elemental analysis ($mg_{chitosan}/g_{sample}$)	Ninhydrin assay ($mg_{chitosan}/g_{sample}$)
1	4% CA–AA	0.68	6.78 ± 0.94	4.613 ± 0.47
2	8% CA–AA	0.69	6.93 ± 0.20	8.15 ± 0.66
3	12% CA–AA	0.77	7.75 ± 0.48	5.73 ± 0.30
4	4% CA	0.64	6.45 ± 0.09	8.44 ± 0.75
5	4% CA–2.3% NaH_2PO_4	1.65	16.53 ± 5.93	15.63 ± 2.18
6	8% CA–2.3% NaH_2PO_4	1.31	13.07 ± 0.38	19.05 ± 0.59
7	12% CA–2.3% NaH_2PO_4	1.20	12.02 ± 0.94	14.77 ± 1.53
8	4% CA–9.2% NaH_2PO_4	1.20	12.02 ± 0.74	10.71 ± 0.16
9	4% CA–18.4% NaH_2PO_4	1.07	10.75 ± 0.50	10.33 ± 1.13
10	4% CA–2.3% NaH_2PO_4–4 h UV	2.68	26.81 ± 0.05	22.01 ± 0.54
11	4% CA–2.3% NaH_2PO_4–8 h UV	2.72	27.19 ± 0.42	23.46 ± 2.84
12	4% CA–2.3% NaH_2PO_4–20 h UV	2.27	22.67 ± 0.12	23.55 ± 1.88
13	8% CA–2.3% NaH_2PO_4–8 h UV	1.61	16.07 ± 1.51	11.59 ± 0.96
14	12% CA–2.3% NaH_2PO_4–8 h UV	1.50	15.02 ± 1.09	16.19 ± 0.77

[a] All the results shown are the mean of three determinations and their standard deviations.
[b] Polymer loading calculated according to Eq. (3).

insufficient to neutralize the carboxylic groups to favour the esterification at increasing CA (Blanchard & Graves, 2002).

As can be observed in Table 2, entries 10–12, the chitosan content increased significantly when UV-irradiation was previously applied to the cotton fibers using 4 wt/vol% of CA. The content of chitosan in the final materials ranged from 22.67 mg/g to 27.19 mg/g according to elemental analysis (Table 2) and from 22.01 mg/g to 23.55 mg/g by the ninhydrin method. The highest incorporation of chitosan was achieved with the 8 h UV-irradiated cotton fiber (entry 11 in Table 2), which is *ca.* 40% more chitosan in the textile than that without irradiation at the same catalyst and CA contents (entry 5 in Table 2). Sionkowska et al. (2006) have reported surface polarity changes on polysaccharide films due to photooxidation caused by the UV-irradiation, as well as the photodestruction of glycosidic bonds and rings with the formation of reactive carboxyl groups. In our case, longer irradiation times than 8 h did not increase the chitosan incorporation, as it is shown in Table 2 (entries 11 and 12). In a related work based on grafting of chitosan films with acrylate has been reported that at increased UV-irradiation doses, a maximum polymer loading is achieved and then decreases (Khan, Ferdous, & Mustafa, 2005). It is worth to mention that there are disadvantages related with the use of UV-irradiation as a method to modify the surface reactivity due to the morphological heterogeneity of the material. Goddard and Hotchkiss (2007) reported that graft polymerization of bioactive materials can be initiated by irradiation, although the UV-light could be blocked by particles on the surface of the material that might affect treatment consistency.

The SEM micrographs of samples with and without UV-irradiation treatment are shown in Fig. 1. It can be observed that the UV-irradiated samples (Fig. 1b and d) displayed morphological changes compared to those non-UV-irradiated ones (Fig. 1a and c). Indeed, fibers treated without UV-irradiation presented relatively high entanglement, whereas previously UV-irradiated fibers were appreciably loose and separated from each other. The lowest entanglement of the cellulose fibers after UV-irradiation might be due to photoablation. This photoablation in UV-irradiated chitosan may induce changes in the material surface, such as foam formation and expansion of the whole material (Sionkowska et al., 2006). As well, the fibers coated with chitosan showed a veil-forming like onto the cellulose fibers as can be seen in the corresponding SEM micrographs (Fig. 1a and b).

The FTIR spectra from samples of the different steps of the method involving previously UV-irradiation, which is described in Section 2.4.3 of the methodology, show the presence of the C=O ester stretching band (1723 cm^{-1}), which points out that the covalent attachment of the chitosan into cellulose underwent through an ester bond formation. The intensity of the carbonyl ester band decreases after the laundry due to removal of non-reacted CA (traces 1–3 in Fig. 2) and its subsequent decrease in the spectrum of the final treated sample (trace 4 in Fig. 2) might be attributable to partial hydrolyses of the formed ester groups under the final acidic conditions, as described in experimental section. The absorption band at 1653 cm^{-1} due to C=O stretching of the secondary amide of chitosan (trace 5 in Fig. 2) is also observed in the FTIR spectra of the treated fibers (1642 cm^{-1}). As well, the representative N—H vibration band at 1593 cm^{-1} assigned to the primary amine of chitosan (trace 5 in Fig. 2) are slightly visible in the spectra of the cellulose cross-linked chitosan samples (traces 1–4 in Fig. 2).

The TGA thermograms shown in Fig. 3 display a weight loss DTG peak of the chitosan sample at *ca.* 300 °C (trace a in Fig. 3), which is similar to that reported by Cardenas and Miranda (2004) for a chitosan with similar molecular weight and acetylating degree. The raw cotton fiber (trace b in Fig. 3) presented a single DTG peak at 350 °C, this temperature lies between the range of 243 °C and

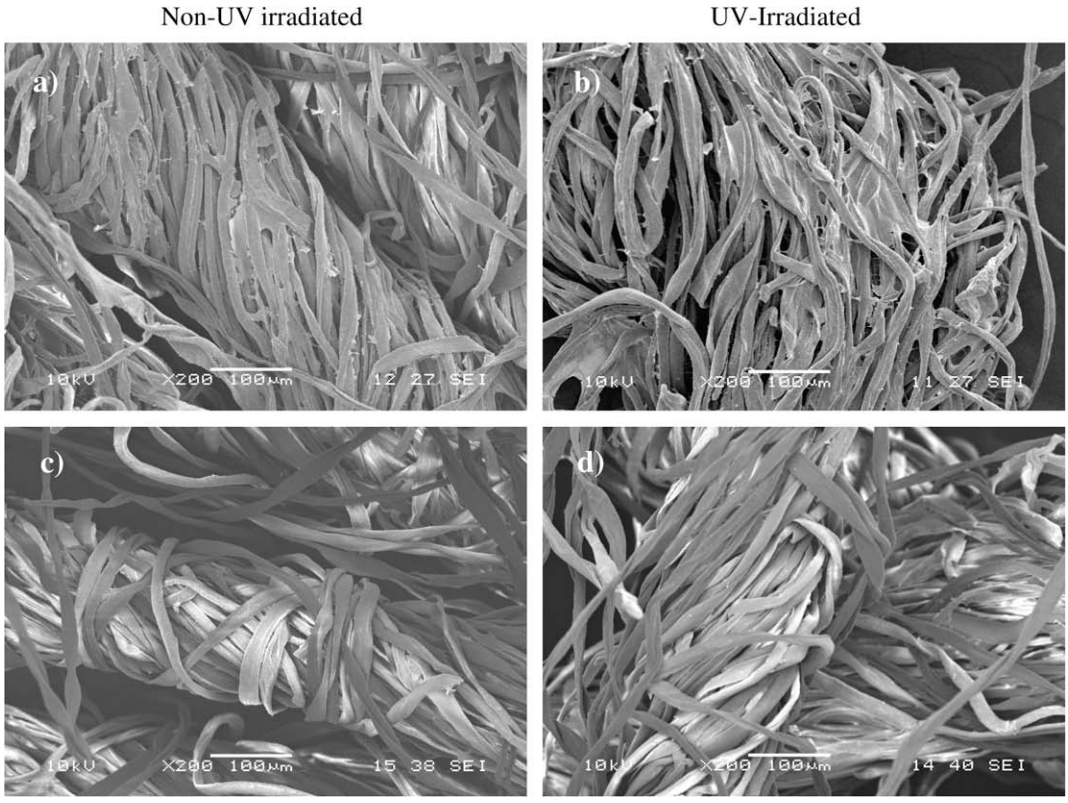


Fig. 1. Scanning electron micrographs of cellulose fibers: (a) chitosan in 4 wt/vol% of CA and 2.2 wt/vol% NaH_2PO_4; (b) chitosan in 4 wt/vol% of CA and 2.3 wt/vol% NaH_2PO_4 over an 8 h UV-irradiated cotton fibers; (c) raw cotton fiber; (d) raw cotton fibers after 8 h of UV-irradiation.

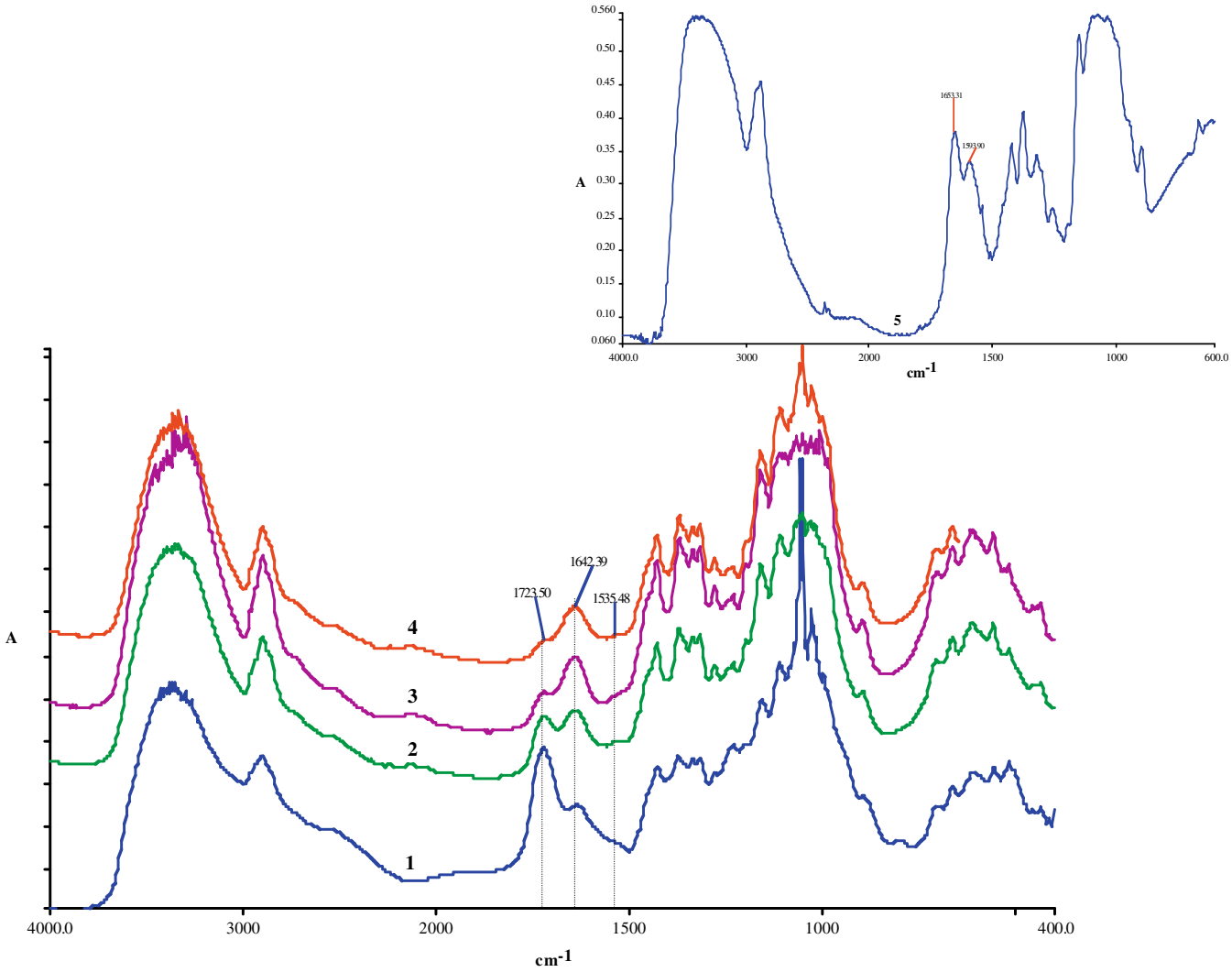


Fig. 2. FTIR spectra during heat-treatment of UV-irradiated cellulose with chitosan using 4 wt/vol% of CA and 2.3 wt/vol% NaH_2PO_4, where FTIR traces: (1) pre-treatment at 70 °C for 5 min; (2) curing at 130 °C for 3 min; (3) after laundry with commercial ionic soap; (4) washed with acetic acid solution (0.1 M); (5) chitosan.

360 °C which correspond to hemicelluloses and alpha celluloses (Sharma, 1996), whereas when chitosan is cross-linked to the previously UV-irradiated cellulose, the DTG peak appears at 370 °C (trace c in Fig. 3) and this would indicate that cellulose becomes thermally more stable.

3.2. Fungistatic and anti-bacterial behaviour of chitosan cross-linked cotton fibers

The results of the determination of the inhibition of the spore germination of *P. chrysogenum* are shown in Fig. 4. In the control samples (raw cotton) the germination started from 10 to 12 h earlier than the functionalized chitosan samples from entries 5 and 11 in Table 2. The effect of chitosan concentration incorporated in the materials over the spore germination process is shown in Fig. 5, where the raw cotton was considered as the control. The spore germination rate (k) of the commercial antimicrobial material was 0.48 h^{-1} and the maximum spore germinated percentage (S_{max}) was 104.9%, whereas with the antimicrobial-finished textile (entry 11 in Table 2) the k was 0.34 h^{-1} and S_{max} of 67.53% (Fig. 5).

The *P. chrysogenum* biomass production with the materials prepared with acetic acid displayed an inhibitory effect against fungi up to X_{max} of 4.6 mg biomass/mL (Fig. 6, entries 1–3 in Table 2), whereas with raw cotton the X_{max} was 9.8 mg/mL. However, the samples with the highest chitosan incorporation inhibited the fungal growth (X_{max}) as much as the commercial textile (Fig. 6, entries 10–12 in Table 2), with a biomass amount less than 4 mg biomass/mL after 120 h of incubation, which was 66% less than the biomass obtained with the raw cotton.

The commercial textile was treated with the quaternary ammonium salt *N*-alkyl dimethyl benzyl ammonium chloride in order to inhibit the bacterial growth by inactivation of enzymes, denaturation of essential proteins of the microorganisms or by breaking cell walls, while, the chitosan mechanisms of inhibition of the microbial growth relies on interactions between charges of the microbial cell walls changing nutrients permeation, i.e., growth restrictions, and eventually causing death (Chan et al., 2001).

The inhibition of fungi, during germination and apical growth, was directly related to the amount of chitosan incorporated in the cross-linked cotton textiles (Figs. 5 and 6). It is noteworthy that the materials tested as antimicrobial were previously laundered and this indicates the covalent attachment of chitosan to the cotton fibers.

Further analyses with *E. coli* were carried out with samples from entries 5 and 11 in Table 2 as they present the highest chitosan incorporation. As it can be seen in Fig. 7, the inhibition rate with the functionalized fibers presented no significant differences with that of the commercial fiber after 43 h of inoculation, and both

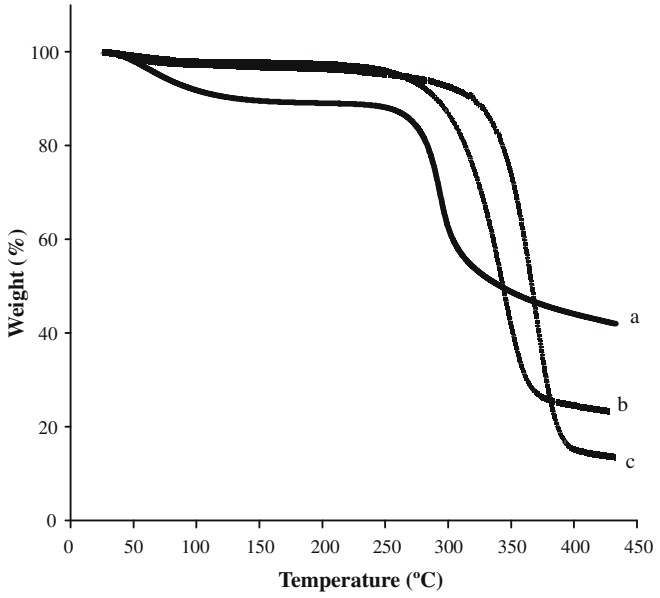


Fig. 3. TGA thermogram traces of several materials: (a) chitosan; (b) raw cotton; (c) chitosan in 4 wt/vol% of CA and 2.3 wt/vol% NaH_2PO_4 over an 8 h UV-irradiated cotton textile.

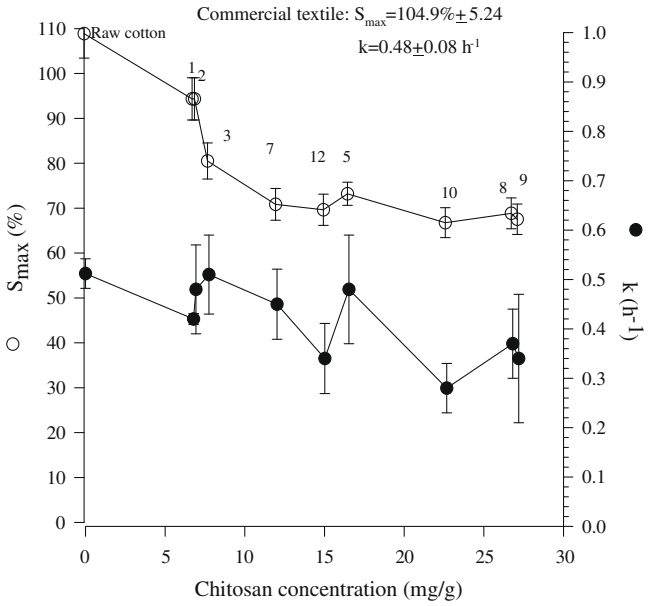


Fig. 5. Specific spore rate germination, k (●), and maximum percentage of germinated spores, S_{max} (○), of *Penicillium chrysogenum* determined with raw cotton, commercial antimicrobial cellulose fiber and several cross-linked cellulose-chitosan concentrations corresponding to entries 1–3, 5, 7, 10–12 and 14 in Table 2. Each point of the graph was estimated by logistic model from time course experimental data of spore germination.

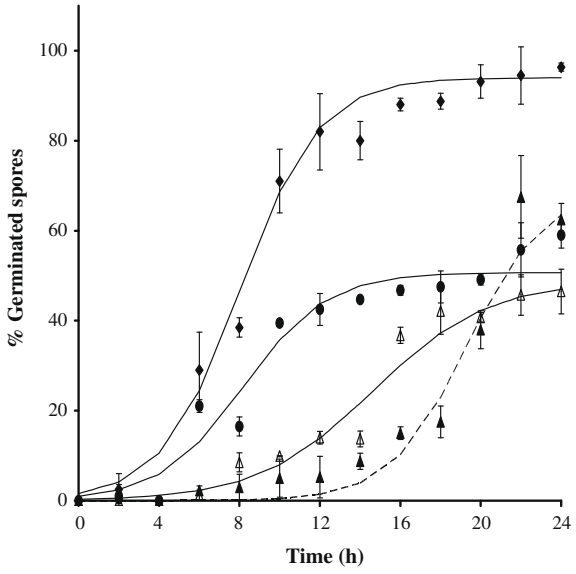


Fig. 4. Spores germination of *P. chrysogenum* determined for several materials: raw cotton fiber (♦); chitosan in 4 wt/vol% of CA and 2.3 wt/vol% NaH_2PO_4 (△); chitosan in 4 wt/vol% of CA and 2.3 wt/vol% NaH_2PO_4 over an 8 h UV-irradiated cellulose fibers (▲) and commercial antimicrobial cellulose fiber (●).

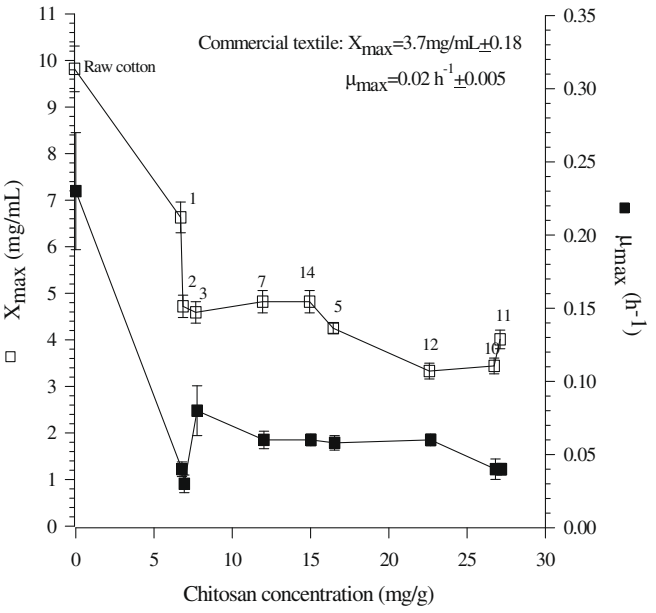


Fig. 6. Specific growth rate, μ_{max} (■) and maximum biomass production, X_{max} (□) of *Penicillium chrysogenum* determined with raw cotton, commercial antimicrobial cellulose fiber and several cross-linked cellulose-chitosan concentrations corresponding to entries 1–3, 5, 7, 10 to 12 and 14 in Table 2. Each point of the graph was estimated by logistic model from time course experimental data of biomass growth.

were significantly inhibitory as compared to raw cotton. The bacterial growth rates (μ) estimated with the materials with highest chitosan content, raw cotton and commercial textile are presented in Table 3, where it is shown a significant difference of *ca.* 0.3 h^{-1} over the cell growth rates between the control and chitosan treated samples.

4. Conclusions

Non-toxic processes have been established for the effective cross-linking of chitosan into cellulose. The use of citric acid as the cross-linking agent and NaH_2PO_4 as catalyst over previously UV-irradiated raw cotton sample gave the highest chitosan incorporation. Ninhydrin method analyses demonstrated that the cross-linking underwent mainly by the hydroxyl group at the C6 of chitosan leaving non-reacted the amino moiety, which plays an important role on the antimicrobial activity. The control against growth of *P. chrysogenum* and *E. coli* was directly related to the amount of chitosan incorporated into the fiber. The materials with the highest chitosan incorporation resulted in similar inhibition of

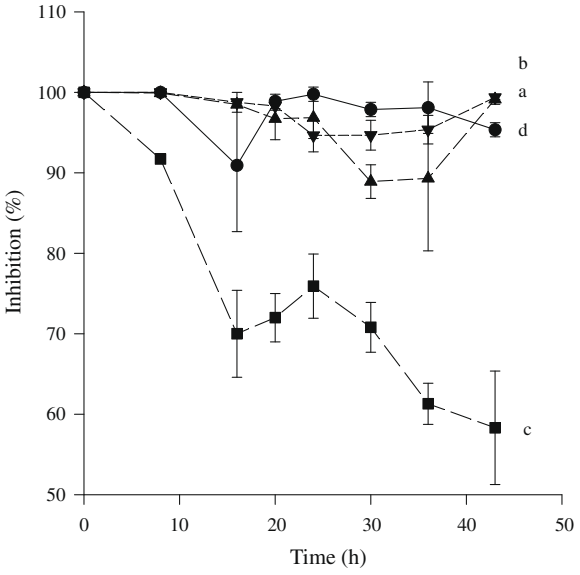


Fig. 7. Percentage inhibition of *E. coli* cells counted in nutrient broth: (a) chitosan in 4 wt/vol% of CA and 2.3 wt/vol% NaH_2PO_4 (▲); (b) chitosan in 4 wt/vol% of CA and 2.3 wt/vol% NaH_2PO_4 over an 8 h UV-irradiated cellulose fiber (▼); (c) raw cotton (■); (d) commercial antimicrobial cellulose fiber (●).

Table 3
Specific growth rates (μ)[a] of *Escherichia coli* in the synthesized cotton fibers estimated by exponential model.

Entry	Treatment	μ (h^{-1})[a]	R^2
	Raw cotton	0.43 ± 0.05	0.986
	Commercial textile	0.10 ± 0.03	0.809
5	4% CA–2.3% NaH_2PO_4	0.10 ± 0.04	0.843
11	4% CA–2.3% NaH_2PO_4–8 h UV	0.10 ± 0.02	0.874

[a] Specific growth rate (μ in h^{-1}) estimated from experimental data of colony forming units by Eq. (2).

fungi and bacterial growth to the commercial textile and significantly higher than raw cotton sample used as a control.

Acknowledgments

The authors gratefully acknowledged to CONACYT for the research funding and for the PhD scholarship to Diana Alonso.

References

Blanchard, E. J., & Graves, E. E. (2002). Polycarboxylic acids for flame resistant cotton/polyester carpeting. *Textile Research Journal, 72*(1), 39–43.
Cardenas, G., & Miranda, S. P. J. (2004). FTIR and TGA studies of chitosan composite films. *Journal of Chilean Chemical Society, 49*(N4), 291–295.
Chan, V., Mao, H. Q., & Leong, K. W. (2001). Chitosan-induced perturbation of dipalmitoyl-*sn*-glycero-3-phosphocholine membrane bilayer. *Langmuir, 17*(12), 3749–3756.
Chang, J. C. S., Foarde, K. K., & VanOsdell, D. W. (1996). Assessment of fungal (*Penicillium chrysogenum)* growth on three HVAC duct materials. *Environmental International, 22*(4), 425–431.
Chung, Y.-S., Lee, K.-K., & Kim, J. W. (1998). Durable press and antimicrobial finishing of cotton fabrics with a citric acid and chitosan treatment. *Textile Research Journal, 68*(10), 772–775.
Coma, V., Sebti, I., Pardon, P., Pichavant, F. H., & Deschamps, A. (2003). Film properties from cross-linking of cellulosic derivatives with a polyfunctional carboxylic acid. *Carbohydrate Polymers, 51*, 265–271.
Curotto, E., & Aros, F. (1993). Quantitative determination of chitosan and the percentage of free amino groups. *Analytical Biochemistry, 211*, 240–241.
Fang, N., Chan, V., Mao, H. Q., & Leong, K. W. (2001). Interactions of phospholipid bilayer with chitosan: Effect of molecular weight and pH. *Biomacromolecules, 2*(4), 1161–1168.
Goddard, J. M., & Hotchkiss, J. H. (2007). Polymer surface modification for the attachment of bioactive compounds. *Progress in Polymer Science, 32*, 698–725.
Kenawy, E. R., Worley, S. D., & Broughton, R. (2007). The chemistry and applications of antimicrobial polymers: A state-of-the-art review. *Biomacromolecules, 8*(5), 1359–1384.
Khan, A. M., Ferdous, S., & Mustafa, A. I. (2005). Improvement of physico-mechanical properties of chitosan films by photocuring with acrylic monomers. *Journal of Polymer and the Environment, 13*(2), 193–200.
Lim, S. H., & Hudson, S. M. (2004). Application of a fiber-reactive chitosan derivative to cotton fabric as an antimicrobial textile finish. *Carbohydrate Polymer, 56*(2), 227–234.
Pacheco, N., Larralde-Corona, C. P., Sepulveda, J., Trombotto, S., Domard, A., & Shirai, K. (2008). Evaluation of chitosans and *Pichia guillermondii* as growth inhibitors of *Penicillium digitatum*. *International Journal of Biological Macromolecules, 43*, 20–26.
Plascencia, J. M., Viniegra, G., Olayo, R., Castillo, M. M., & Shirai, K. (2003). Effect of chitosan and temperature on spore germination of *Aspergillus niger*. *Macromolecular Bioscience, 3*(10), 582–586.
Prochazkova, S., Vårum, K. M., & Østgaard, K. (1999). Quantitative determination of chitosans by ninhydrin. *Carbohydrate Polymers, 38*, 115–122.
Rabek, J. F. (1996). *Photodegradation of polymers, physical characteristics and applications*. Berlin, Germany: Springer-Verlag. pp. 340–346.
Schiffman, J. D., & Schauer, C. L. (2007). Cross-linking chitosan fibers. *Biomacromolecules, 8*, 594–601.
Sharma, H. S. S. (1996). Compositional analysis of neutral detergent, acid detergent, lignin and humus fractions of mushroom compost. *Thermochimica Acta, 285*(2), 211–220.
Sionkowska, A., Kaczmarek, H., Wisniewski, M., Skopinska, J., Lazare, S., & Tokarev, V. (2006). The influence of UV irradiation on the surface of chitosan films. *Surface Science, 600*, 3775–3779.
Sun, S. W., Lin, Y. C., Weng, Y. M., & Chen, M. J. (2006). Efficiency improvements on ninhydrin method for amino acid quantification. *Journal of Food Composition and Analysis, 19*, 112–117.
Takai, K., Ohtsuka, T., Senda, Y., Nakao, M., Yamamoto, K., Matsuoka, J., et al. (2002). Antibacterial properties of antimicrobial-finished textile products. *Microbiology and Immunology, 46*(2), 75–81.
Tharanathan, Rudrapatnam N., & Kittur, Farooqahmed S. (2003). Chitin – the undisputed biomolecule of great potential. *Critical Reviews in Food Science and Nutrition, 43*(1), 61–87.
Yang, C. Q. (1993a). Effect of pH on the nonformaldehyde durable press finishing of cotton fabrics: FT-IR spectroscopy study. Part I. Ester cross-linking. *Textile Research Journal, 61*, 420–430.
Yang, C. Q. (1993b). Effect of pH on nonformaldehyde durable press finishing of cotton fabric: FT-IR spectroscopy study. Part II. Formation of the anhydride intermediate. *Textile Research Journal, 63*(12), 706–711.
Zhang, Z., Chen, L., Ji, J., Huang, Y., & Chen, D. (2003). Antibacterial properties of cotton fabrics treated with chitosan. *Textile Research Journal, 73*(12), 1103–1106.