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Susceptibility of dibutyryl chitin and regenerated chitin fibres to deacylation and depolymerization by lipases

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Abstract

Dibutyryl chitin obtained by esterification with butyric anhydride and regenerated chitin obtained from dibutyryl chitin by saponification, both in the form of wet-spun fibres and non-wovens, were examined by infrared spectrometry and X-ray diffraction spectrometry. Chitin fibres and chitosan fibres were also studied for comparison, and found to maintain the XRD spectral features of the parent chitin and chitosan. On the opposite, DBC fibres and non-woven exhibited depressed crystallinity, the peak at 0.46–0.47 nm, typical of chitin, being hardly detectable, while the one usually at ca. 1.00 nm was present at ca. 1.20 nm. Both DBC fibres and non-woven were highly oriented. When exposed to porcine pancreatic lipase or wheat germ lipase, the DBC fibres gained improved crystallinity with peaks at 1.14–1.18 and 0.41 nm, due to partial regain of chitin structure as a consequence of partial enzymatic removal of butyryl groups, as confirmed by ATR-FTIR. The RC fibres exhibited broad XRD peaks at 0.96 and 0.36 nm; sharper peaks at 0.34, 0.46–0.49 and 0.96 were observed after exposure to lipases, due to removal of a disordered polymer fraction susceptible to the unspecific enzymatic depolymerization. In fact the RC fibres were found to have 8% lower degree of acetylation compared to parent chitin, as a consequence of the alkaline regeneration treatment. In conclusion, these modified chitins are scarcely susceptible to degradation by lipases (besides to lysozyme, as already reported in the literature); therefore their biochemical significance in wound management seems limited. They, however, appear to be the ideal textile materials for providing mechanical support to freeze-dried chitosan sponges having amply documented activity in wound healing, and for the preparation of specialty textiles.

Keywords: Chitin; Dibutyryl chitin; Regenerated chitin; Textile fibre; Textile non-woven; Lipase

1. Introduction

Chitin is soluble in a few corrosive and toxic solvents such as chloroalcohols, chloroacetic acid, formic acid, methylene chloride, hexafluoroisopropanol, and a couple of aprotic solvents, dimethylacetamide and *N*-methyl-2-pyrrolidone containing 7% LiCl; also lithium thiocyanate is a solvent (Austin, 1975; Austin & Brine, 1977; Capozza, 1976; Rutherford & Austin, 1978; Tokura, Yoshida, Nishi, & Hiraoki, 1982).

By converting chitin into chitin xanthate it was possible to regenerate the chitin in form of fibres after extrusion (Balassa & Prudden, 1978; Noguchi, Tokura, & Nishi, 1978; Thor, 1939; Thor & Henderson, 1940). Unfortunately the fibres have scarce tenacity and this characteristic property prevented their use, particularly as suture threads. On the other hand, filaments were conveniently produced as chitosan floc by Minami, Okamoto, Hamada, Fukumoto, & Shigemasa, (1999), who also manufactured the chitosan cotton currently commercialized as Chitipack-C®, (Eisai Co., Tokyo).

The difficulties inherent to the production of chitin-based textiles and non-woven were also circumvented by incorporating chitin fine powders into a reinforcing material such as a non-woven fabric of polyethyleneterephthalate, Chitipack-P® (Eisai Co., Tokyo), or preparing freeze-dried

Abbreviations: DBC, dibutyryl chitin; RC, regenerated chitin from DBC; XRD, X-ray diffraction spectrometry; ATR-FTIR, attenuated total reflection-Fourier transform infrared spectrometry; PPL, porcine pancreatic lipase; WGL, wheat germ lipase.

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sponges from chitin suspensions, Chitipack-S® (Eisai, Co., Tokyo) (Minami et al., 1999). Beschitin-W® (Unitika, Tokyo) is a non-woven fabric of chitin (Hirano, 1996). Sandford (1992) announced a chitosan-based dressing that today is commercial, Tegasorb® (3M Co., Minneapolis).

These materials have been found to be efficient filling agents for surgical tissue defects, because they are degraded and resorbed in situ and finally replaced by normal tissue, as a point of difference from conventional filling agents made of vinyl chloride, styrene or silicone, totally deprived of biochemical significance.

Early reports on the development of chitin and chitosan in the preparation of materials suitable for the manufacture of the said wound dressing were those by Kaifu, Nishi, Komai, Tokura, and Somorin (1981); Takai et al. (1989); Tokura et al. (1982).

The spinning of chitosan is easy, involving simply a chitosan acetate salt solution and a coagulation bath (5% NaOH). Chitosan in yarn form was then optionally acetylated with acetic anhydride thus regenerating chitin (East, McIntyre, & Qin, 1988). Overall, however, the re-acetylated chitin fibres were inferior to those made by direct spinning of chitin dissolved in DMA-LiCl.

A porous chitin gauze was made by Kifune (1992) and was found useful for intranasal medication after surgery. A review on chitosan and chitin fibres has been published (Agboh & Qin, 1997).

Dibutyryl chitin, DBC, an ester of chitin, is easily soluble in common organic solvents and has both film- and fibre-forming properties suitable for manufacturing an assortment of DBC materials for medical applications (Szosland, 1996, 1997; Szosland & East, 1995). It was found also that the treatment of finished materials made of DBC, carried out under mild alkaline conditions, led to chitin regeneration (Szosland, 1996).

Preliminary investigations of the biological properties of DBC and RC materials, carried out in vitro and in vivo according to the European standards EN ISO 10993 (Biological evaluation of medical devices), showed good biocompatibility of both polymers and ability to accelerate wound healing (Pielka et al.,2003).

The degradability of chitin fibres by lysozyme has been addressed by Tokura, Nishi, Nishimura, & Somorin (1983) who found that chitin fibres were not degraded by lysozyme whilst carboxymethyl chitin was extensively degraded (80% weight loss in 2 h). Butyl chitin, dihydroxypropyl chitin and glycol chitin fibres were sparingly hydrolysed (2% weight loss in 2 h). Therefore, the substitution of chitin with hydrophobic groups confers degradability to a modest extent. Results were confirmed by Shigemasa, Sashiwa, Saimoto, & Tokura (1992).

The hydrolytic activity of lipase on crab α-chitin and squid β-chitin has been recently examined by Sashiwa, Fujishima, Yamano, Kawasaki, Nakayama, Muraki, Mongkol, Pichyangkura, & Aiba (2003) who believe that chitinases and β-*N*-hexosaminidases are present as impurities in lipases. They have found that lipase has much higher exochitinase activity than cellulases (9.8 vs 0.18–1.4) in addition to endochitinase activity (2.7 vs 3.7–12.3). They have also sustained the view that some enzymes in admixture enhance reciprocally their activities.

On the other hand, it is known that lipases of various origins depolymerize chitosans (Muzzarelli, Cosani, & Terbojevich, 1996; Muzzarelli, Xia, Tomasetti, & Ilari, 1995; Qin, Du, Xiao, Li, & Gao, 2002; Sashiwa et al., 2002), and while certain authors sustain the view that their unspecific activity on chitosans is due to accompanying chitosanases, other authors are convinced that the unspecific activity is justified by the simplicity of the enzymatic hydrolytic mechanism and similarity of the active sites (Fu, Wu, Chang, & Sung, 2003; Roy, Sardar, & Gupta, 2003). In humans, chitinolytic activity may be exerted by *N*-acetylglucosaminidase or chitotriosidase (Barone, Simpore, Malaguarnera, Pignatelli, & Musumeci, 2003; Muzzarelli, 1997). It is, however, expected that exogenous chitinous materials are hardly degraded in the human body.

When dealing with DBC, however, the chemical nature of the modified chitin, an ester, suggests that it would be susceptible to enzymatic attack by lipases insofar as the removal of butyryl groups is concerned.

Scope of the present report is therefore the assessment of the enzymatic susceptibility of DBC and RC to lipases and consequences on their polymer structure.

2. Experimental

2.1. Chitins

The chitins were those preferred by Tokura, Nishi, and Nishimura (1983), for the preparation of chitin and chitosan fibres (Queen crab chitin, prepared according to Hackman), and by Szosland (1997) for the preparation of DBC and RC fibres (*Euphausia superba* krill chitin), according to their respective methods. The krill chitin had intrinsic viscosity 13.33 dL/g determined in dimethylacetamide +5% LiCl solutions at 25 °C, viscosity average molecular weight 287 Kda and degree of crystallinity 65.3%; it was supplied by Sea Fisheries Institute, Gdynia, Poland.

2.2. Synthesis of dibutyrylchitin

The acylation mixture was prepared, by pouring perchloric acid into butyric anhydride at ca. −12 °C. Some butyric acid was formed, due to water present in ca. 70% $HClO_4$. The acylation mixture was added slowly to the chitin powder placed in the reactor cooled in a bath (ca. 0 °C during 30 min, ca. 20 °C later). Reagents were mixed slowly. During acylation chitin reacted with the rest of butyric anhydride (acylation agent) in the presence of perchloric acid (catalyst).

The chitin esterification was carried out at ca. 20–30 °C during 3–4 h using reagents and catalyst in molar ratios chitin: Bu_2O:$HClO_4$ = 1:4:1. The exothermic reaction ended when the temperature in the reactor became equal to the temperature of the cooling bath (ca. 20 °C). Diethyl ether was added and the solid product was recovered by filtration and washed with diethyl ether for quick removal of butyric acid and excess butyric anhydride. The consumption of diethyl ether was ca. 500 ml/100 g of the product. Then the product was washed with water to pH 7, collected by filtration and dried at ca. 100 °C. Dry product was placed in acetone to allow dibutyrylchitin to dissolve. After removal of any insoluble material, DBC was precipitated with water (yield 90%). Weight average molecular weight values, determined by size exclusion chromatography coupled with light scattering and viscometry, were in the range 120–200 KDa (Szosland, 1996, 1997; Szosland & East, 1995; Szosland et al., 2001).

2.3. Characteristics of DBC

Average results of elemental analysis of DBC gave: C = 55.80% (calculated 55.98%), H = 7.51% (calculated 7.29%) and N = 4.10% (calculated 4.08%) and suggested that the degree of esterification was very close to 2; intrinsic viscosity 1.41 dL/g in acetone at 25 °C; intrinsic viscosity 2.17 in dimethylacetamide at 25 °C; weight average molecular weight ca. 170 KDa; degree of crystallinity (when in form of flakes) 47.2%. The fibres were prepared by wet spinning, by using a 16% solution of DBC in dimethylformamide; the non-wovens were prepared from 6-cm fibres by the needle-punching technique (Szosland et al., 2001).

The ^{1}H NMR spectrum of DBC was recorded using a Brucker AM 400 spectrometer. Deuterated chloroform, $CDCl_3$ was used as a solvent. In the ^{1}H spectrum both substituted hydroxyl groups in the secondary position 3, at 5.0 ppm, and in the primary position 6, at 4.3 ppm, gave well separated signals. This applies to both protons in CH_3 and CH_2 groups. The proton signals of *N*-acetyl group of the original chitin are present in the spectrum as a singlet placed at 1.9 ppm.The intensity ratio of signals of CH_3 group, coming from the butyryl substituent, to the signal intensity of *N*-acetyl group, derived from the spectra, amounts to 1:2. According to NMR investigation the reaction product is in fact dibutyryl chitin. Both signals of amide group: CH_3–CO at 2.0 ppm and NH at 6.1 ppm indicate that more than 90% of N-acetyl groups of the original chitin are present in DBC.

In the FTIR spectrum of DBC there is no band at ca. 3500 cm^{-1} assigned to hydroxyl groups, because the latter are esterified. There are new strong bands at 1740 cm^{-1} and around 1450 cm^{-1} characteristic of the esters of fatty acids. Furthermore, bands of stronger absorption appear at around 2900, 790 and 740 cm^{-1} corresponding to aliphatic groups $>CH_2$ and $-CH_3$, whose content in DBC is higher than in chitin.

2.4. Preparation of regenerated chitin

The DBC fibres were immersed in 1.25 M aqueous NaOH solution at 50 °C and allowed to be hydrolysed for definite periods of time up to 150 min; then the samples were washed several times with water to remove alkali, dried and weighed; then they were treated with acetone to extract unreacted DBC and dried and weighed again. The intrinsic viscosity of the residuum was determined in the DMA + 5% LiCl solutions at 25 °C.

2.5. Treatment with lipases

Lipase solutions were prepared by dissolving the desired lipase (100 mg) in 100 ml of a 0.5 g/l NaN_3 aqueous solution, final pH 6.0. The samples (ca 200 mg) were introduced in 40 ml of the enzyme solutions and kept on a rolling bar machine for 4 weeks. The lipases (EC 3.1.1.3) were crude porcine pancreas lipase L-3126 (PPL), and wheat germ lipase L-3001 (WGL), both supplied by Sigma-Aldrich, Milan, Italy.

2.6. Instruments

X-Ray diffraction measurements on powder samples were performed with the Bruker AXS General Area Detector Diffraction System (GADDS) equipped with a two-dimensional (2D) gas-filled sealed multiwire detector (scattering-angle resolution of 0.02°). Monochromatized Cu Kα radiation (λ =0.154 nm) was used. The powder samples were placed in 0.8-mm diameter Lindemann glass capillaries. The sample/detector distance was 10 cm. The intensity vs scattering-angle spectra were obtained after proper radial average of the measured 2D diffraction patterns. Data for FG90 chitosan: 8.22, 19.30° 2θ. Basic structural data can be found in Cairns et al. (1992).

A Perkin Elmer Spectrum GX FT-IR spectrometer equipped with a Perkin Elmer Multiscope system infrared microscope (MCT-SL detector) was used to record Attenuated Total Reflection, ATR, spectra. The microscope was equipped with a movable 75 × 50 mm *X*–*Y* stage. In some cases it was necessary to adopt the following procedure: small amounts of the sample, cooled in liquid nitrogen, were ground with KBr and the spectra were obtained by using a Spectra Tech. Diffuse Reflectance (DRIFT) accessory. In both cases, the spectral resolution was 4 cm^{-1}. The absorption spectra were the results of 16 scans. Treatments of the data were achieved with a Perkin Elmer Spectrum and with a Grams/32 Galactic Corp. software package. Data for chitin: typical bands at 3436 (NH and OH stretching), 2924 (CH stretching), 1656 (amide I CO stretching), 1623, 1561 (amide II, NH deformation in the CONH plane), 1424 (CH deformation), 1377 (C–CH3 amide stretching), 1314 (amide III and >CH2 wagging), 1158 (COC bridge stretching), 1070 (C–O–C stretching in ring), 1030 (CO stretching), 894 (beta linkage), 700

and 599 cm^{-1}. Spectra were recorded in the ATR mode for the DBC and RC fibres as such, and for the DBC samples treated with wheat germ lipase or porcine pancreatic lipase. Details on the ATR technique can be found in Snabe & Petersen (2002).

3. Results and discussion

3.1. ATR-FTIR spectra for DBC

The spectra have been normalised for comparison at 2880 cm^{-1} (Fig. 1). The untreated DBC fibre exhibits the typical butyrate ester band at 1750 cm^{-1}, accompanied by the band at 1675 cm^{-1} containing a minor contribution from ester and assigned to the amide 1656 cm^{-1} of chitin (shoulder clearly detected) and 1555 cm^{-1} assigned to amide of chitin. A further butyrate ester contribution is detected around the 1200 cm^{-1} value.

The spectrum for the DBC fibre treated with pancreatic lipase shows a depressed 1750 cm^{-1} band, hardly distinguishable from the next 1656 cm^{-1} band. What is a shoulder in other samples here appears as a large band at ca. 1650 cm^{-1} indicative of a smaller contribution by ester. All this is indicative of partial removal of butyryl groups from DBC. Also the 1200 cm^{-1} region was less defined. This spectrum also shows more important contributions at 1630 and 1410 cm^{-1} (amide bands) indicative of decreased ester/amide ratio. This becomes even more apparent when the spectra are normalised at 1732 cm^{-1} (Fig. 2).

The spectrum for DBC treated with wheat germ lipase is most similar to the intact DBC fibre spectrum, showing poor efficacy of wheat germ lipase on this substrate.

All of these spectra contain enhanced alkyl group bands in the interval 2800–3000 cm^{-1} due to the high esterification degree. Even in this range, a difference is visible between the pancreatic lipase treated DBC fibre and the other two samples.

3.2. ATR-FTIR spectra for regenerated chitin

The ATR-FTIR spectra recorded on regenerated chitin (RC) have been normalised at 3107 cm^{-1} (Fig. 3). These spectra clearly exhibit all features of chitin spectra and it can be stated first of all, that the regeneration process in alkali actually modified the DBC to chitin and not to chitosan. The chitin characteristics bands listed above are all present, in particular the bands 1656 and 1561 are in the correct ratio for genuine chitin. Having been normalised at 3107 cm^{-1}, the spectra can be compared in terms of sharpness and intensity of the bands, and actually both spectra for the lipase treated DBC are more detailed than the untreated spectrum.

The intensity ratios 1555/2880 were: for RC fibre 0.79; for RC-PPL 0.86; for RC-WGL 0.83; for genuine shrimp chitin 0.86; for genuine krill chitin 0.85. The ratio for the RC fibre was significantly different from the other samples and the two genuine chitins, thus indicating a lower degree of acetylation, that is, however, impossible to determine by FTIR (Muzzarelli, Tanfani, Scarpini, & Laterza, 1980).

3.3. X-Ray diffraction analysis

All samples exhibited crystalline features related to those of chitin.

3.3.1. Chitin fibres and chitosan fibres

Fig. 4 shows the XRD patterns of chitin fibres; for comparison purposes the XRD pattern of chitosan fibre is also reported. The spectrum of chitin fibre exhibits broad

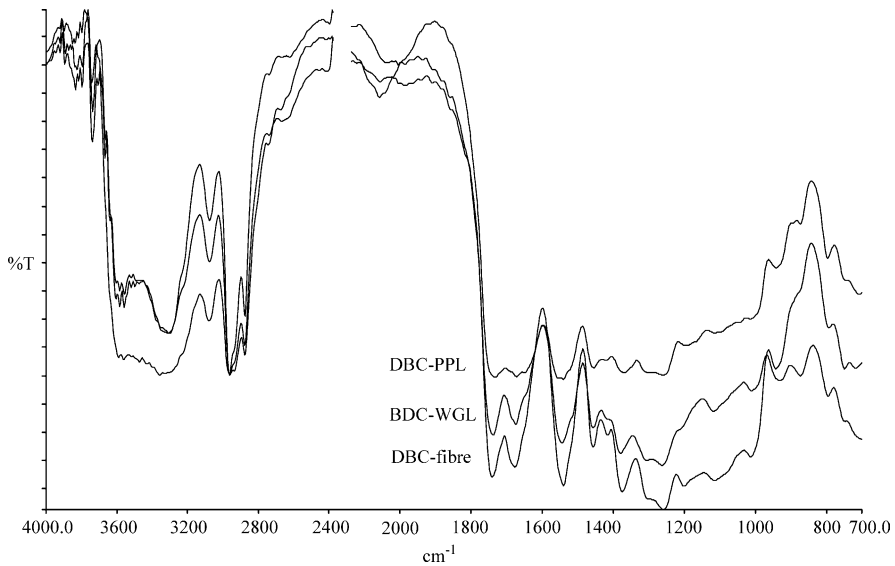

Fig. 1. ATR-FTIR spectra for DBC fibres, for the wheat germ lipase treated DBC, and for the porcine pancreatic lipase treated DBC. The spectra have been normalized at 2880 cm^{-1}.

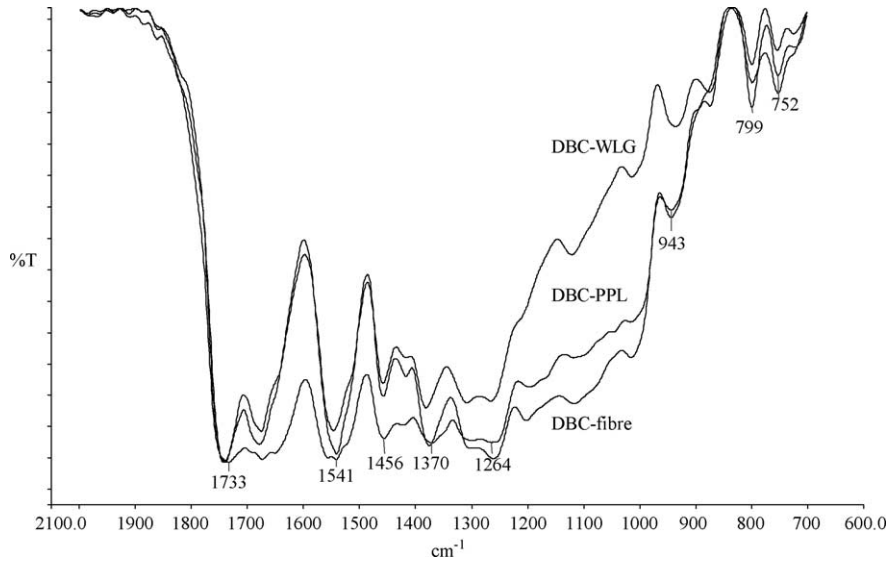

Fig. 2. Detail of the spectra in Fig. 1, normalized at 1732 cm^{-1}, providing evidence of alterations at wavenumbers close to 1600 and 1420.

peaks at 0.34, 0.45, 0.50 and 1.09 nm with a shoulder at 0.71 nm. In the chitosan fibre spectrum, peaks were found at 0.45, 0.88 and 2.93 nm.

3.3.2. Dibutyryl chitin fibre

Fig. 5A shows the XRD pattern of DBC fibre, that is substantially different from that of chitin fibre in Fig. 4, a main feature being a strong and moderately sharp peak in the small-angle region at 1.23 nm and a weak diffuse broad halo centred about 0.42 nm. Such pattern is consistent with a structural organization of the polymeric chains involving one-dimensional (1D) lamellar-type ordering in the direction of the long axis (longitudinal direction). The periodicity at 1.23 nm gives the thickness of the layer whereas the 0.44 nm spacing corresponds to the average inter-chain distance. This pattern is similar to the characteristic spectrum observed in the smectic phase of liquid crystalline polymers (Davidson & Levelut, 1992). However, the moderate sharpness of the small-angle diffraction peak is consistent with longitudinal positional ordering extending over a relatively short space scale. This length scale is measured by the longitudinal correlation length ξ, which was estimated through the formula:

$$\xi = \frac{1.59\lambda}{\Delta(2\theta)\cos\theta_B} \quad (1)$$

where $\lambda = 0.154$ nm, $\Delta(2\theta)$ is the full width at half maximum (FWHM) of the small-angle Gaussian diffraction peak (at $d = 1.23$ nm) and θ_B is the corresponding Bragg

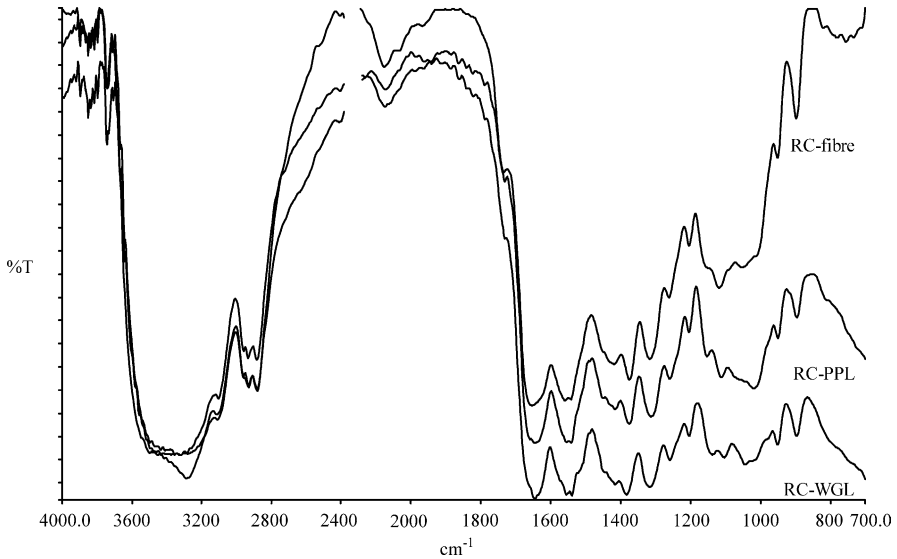

Fig. 3. ATR-FTIR spectra of RC fibres (upper curve), for the wheat germ lipase treated RC (lower curve), and for the porcine pancreatic lipase treated RC (middle curve). The spectra have been normalized at 3107 cm^{-1}.

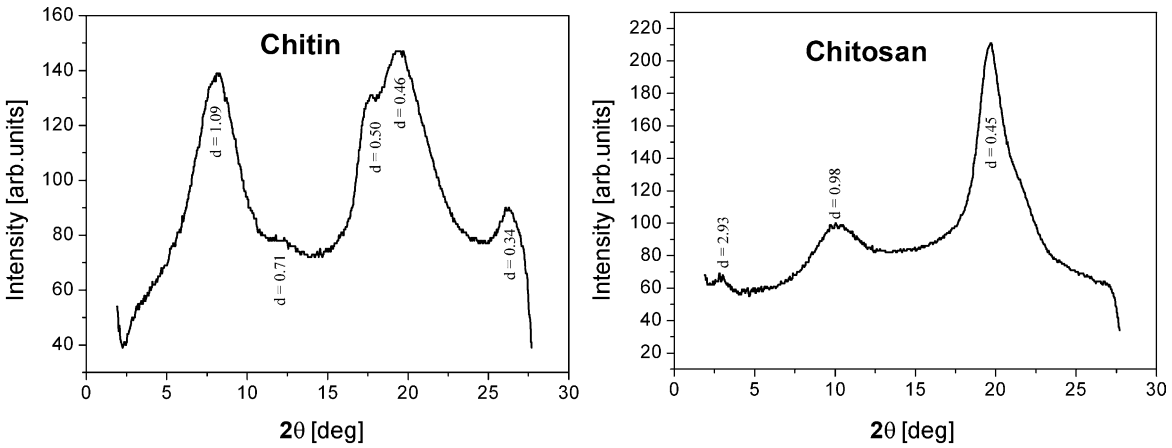

Fig. 4. X-Ray diffraction spectra of chitin fibres and chitosan fibres. The numbers indicate the d-spacing of the peaks in nm.

angle (Francescangeli, Laus, & Galli, 1997). Thus we found $\xi = 0.7$ nm.

3.3.3. Lipase-treated DBC fibres

The XRD patterns for the lipase-treated fibres DBC-PPL and DBC-WGL are shown in Fig. 5C and D. A comparison with the pattern of DBC fibre reveals some similarities. In fact, in both cases a relatively sharp peak is observed in the small angle region at 1.19 nm (very close to the 1.23 nm for DBC) accompanied by a wide and diffuse halo centred at about 0.42 nm. For both materials the width of the small-angle diffraction peak is similar to that of the DBC fiber, thus indicating correlation lengths again on the order of 0.7 nm.

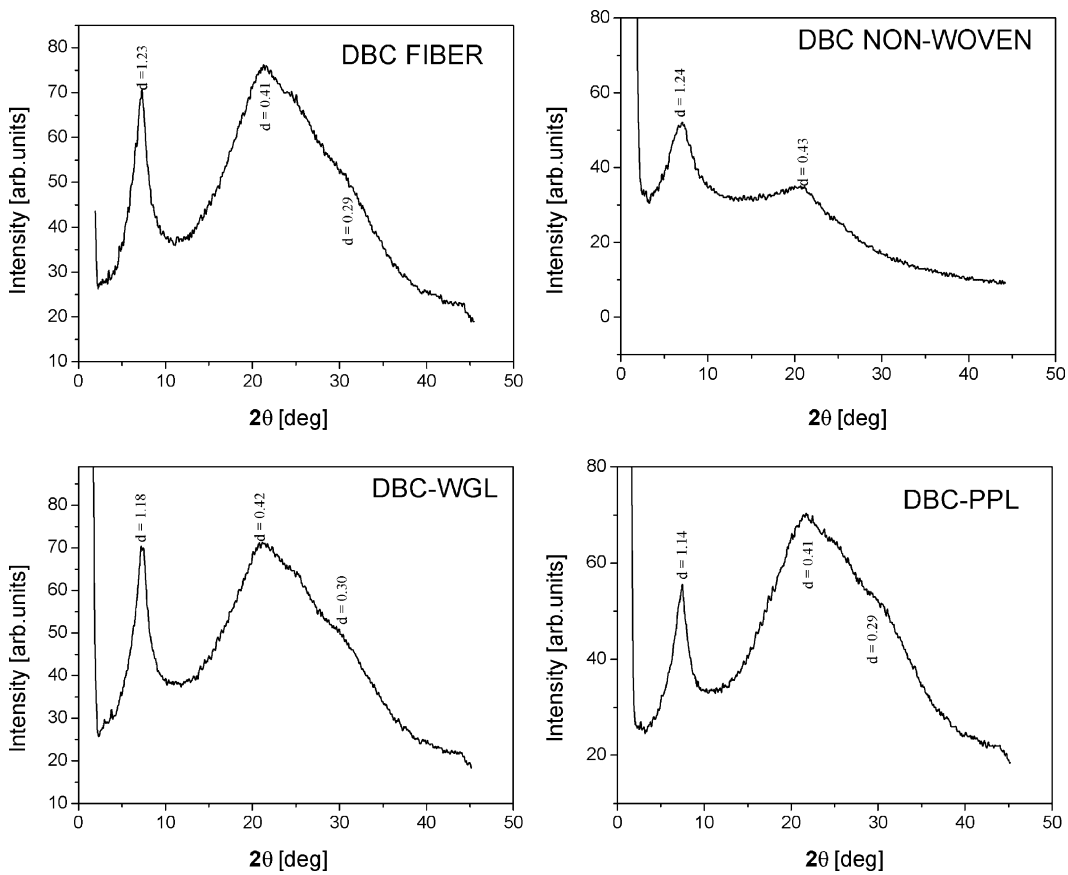

Fig. 5. X-Ray diffraction spectra of dibutyryl chitin fibres; dibutyryl chitin non-woven; wheat germ lipase-treated DBC fibres, and porcine pancreatic lipase-treated DBC fibres. The numbers indicate the d-spacing of the peaks in nm.

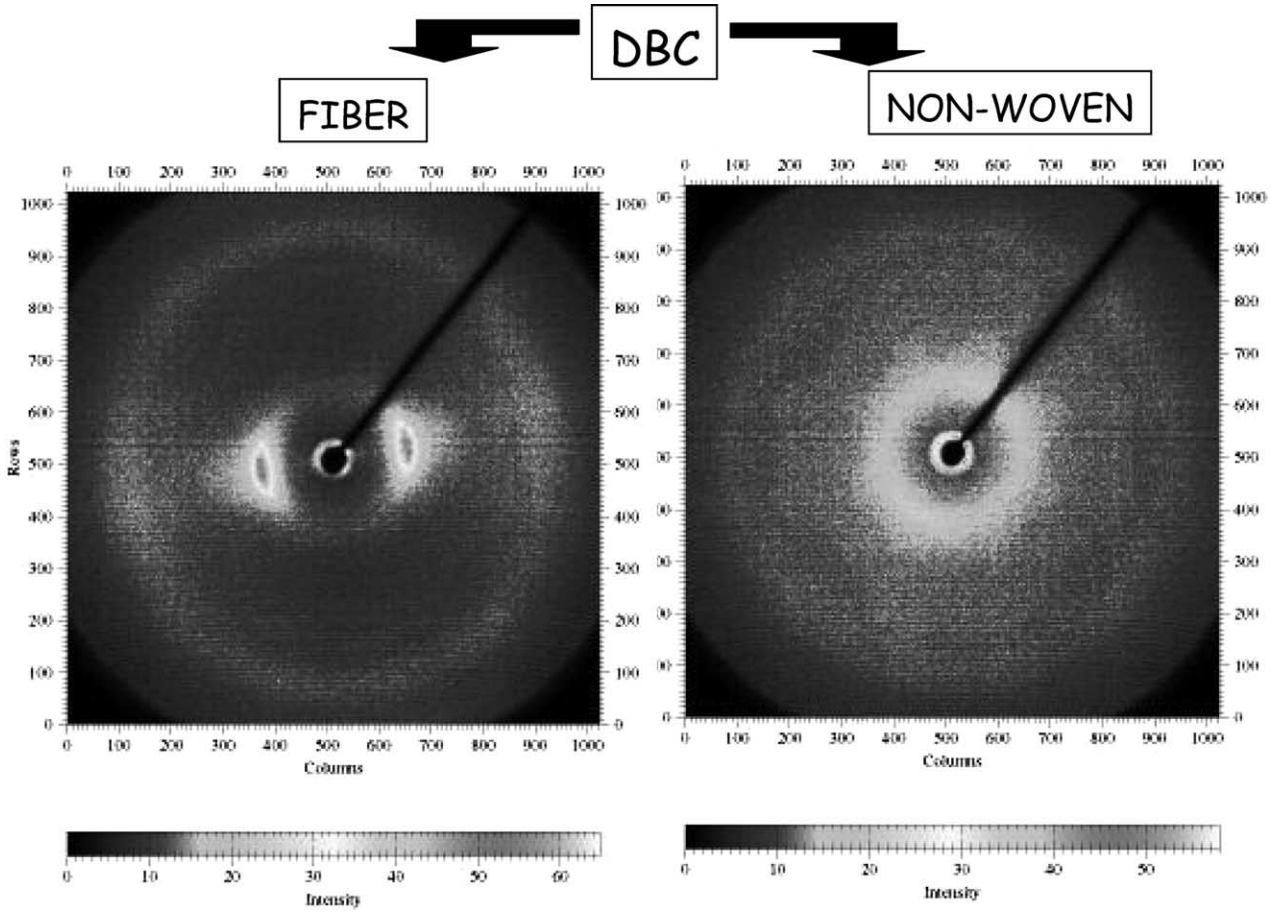

Fig. 6. Two-dimensional XRD patterns of DBC fibre and non-woven DBC, showing different orientation degrees.

However, in addition to these features, a couple of weak diffuse reflections are present in the wide angle region, superimposed as shoulders to the above strong diffuse halo at 0.42 nm; the presence of these two signals is consistent with the onset of an increased amount of long-range order normal to the fibre axis, possibly promoted by the hydrolytic action of the lipase, even though exerted to a limited extent. In fact, lipases are esterases that should certainly be able to remove butyl groups from the fibre surface, thus forming a better spatial organization of the chain normally to the long axis.

3.3.4. Non-woven dibutyryl chitin

The XRD spectrum of the non-woven DBC exhibits some similarities with the spectrum for DBC fibre (Fig. 5), except for the reduced sharpness of the low-angle peak, which means lower degree of lamellar order, and less pronounced wide-angle signal. The increase of the FWHM of the low-angle peak corresponds to a reduction of the correlation length to 0.37 nm, a value around one half that found in the DBC fiber, which is consistent with the non-woven nature of the material. Moreover, a comparison between the two-dimensional XRD patterns of DBC fibre and non-woven DBC in Fig. 6, indicates that the latter is remarkably less oriented than the former.

3.3.5. Regenerated chitin fibre and non-woven regenerated chitin

Fig. 7A and B show the XRD pattern of the regenerated chitin fibre and the non-woven regenerated chitin, respectively. The former exhibits weak and diffuse peaks, some of which not well resolved, centred at 0.34, 0.70, 0.47 and 0.99 nm indicating a poorly oriented semi-crystalline structure. Also the pattern of plain non-woven RC in Fig. 7B is of semi-crystalline nature and most similar to that of the RC fibre: in fact, peaks at 0.34, 0.47 and 0.98 nm accompanied by shoulders at 0.71 and 0.51 nm are observed.

A comparison between the two figures clearly shows that the degree of crystallinity (i.e. the volume fraction of the crystalline material over the total volume crystalline + amorphous) in non-woven RC is much higher than in the RC fiber. However, the non-woven RC is remarkably less oriented than the RC fiber, as shown in Fig. 8. The average size of the crystalline domains of these materials was estimated from the FWHM of the Bragg peaks by means of the Scherrer formula (Cullity, 1978). Consistent

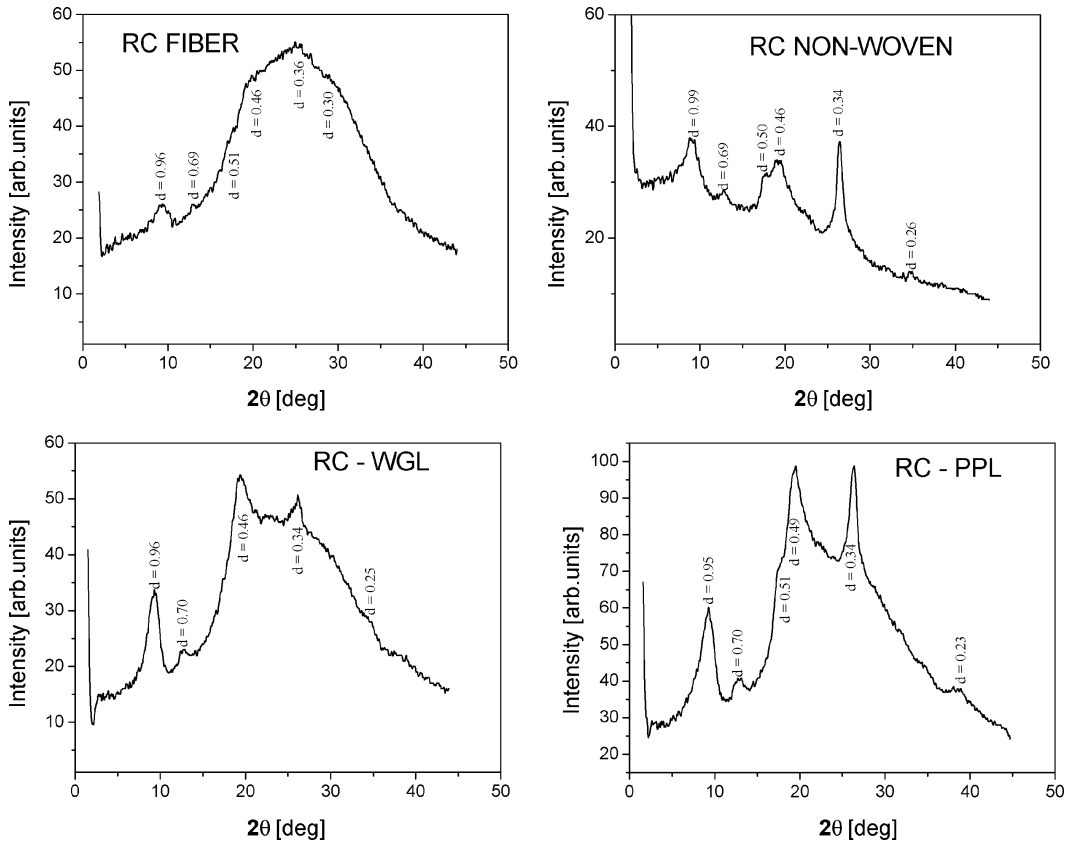

Fig. 7. X-Ray diffraction spectra of regenerated chitin fibres; regenerated chitin non-woven; wheat germ lipase-treated RC fibres, and porcine pancreatic lipase-treated RC fibres. The numbers indicate the d-spacing of the peaks in nm.

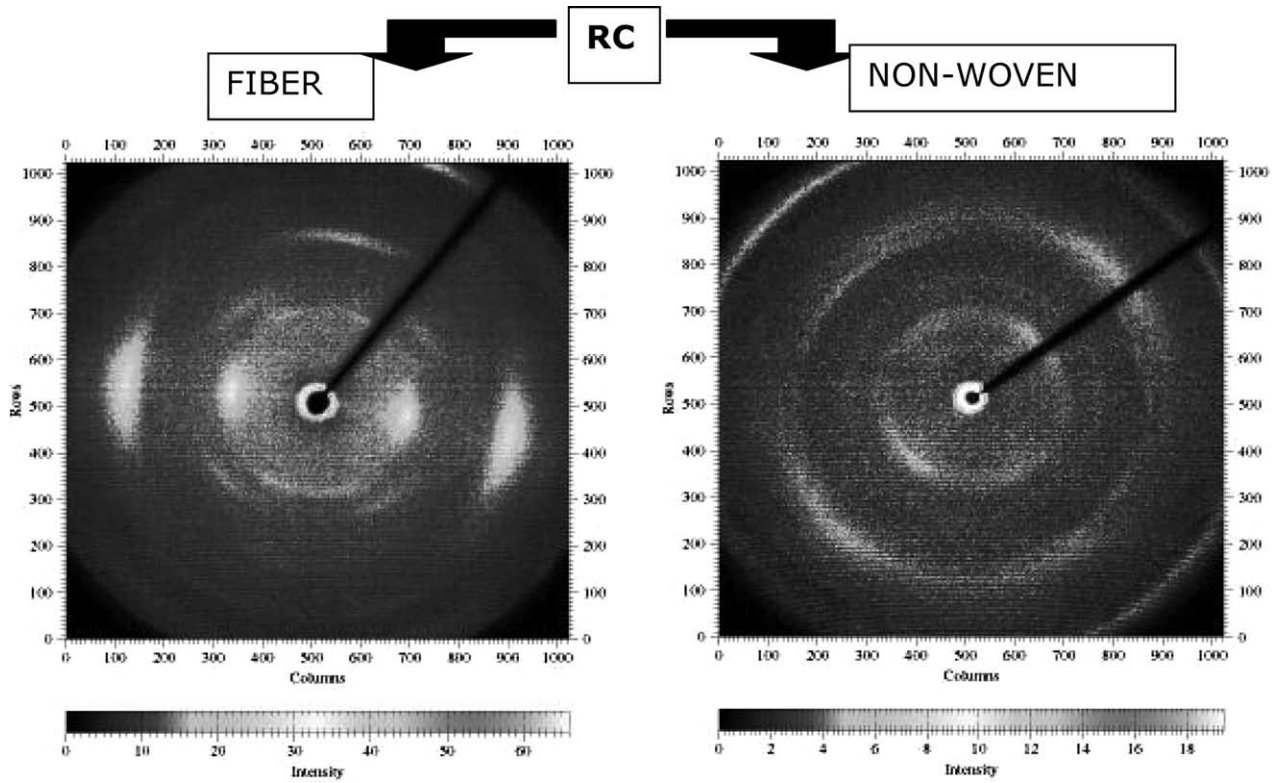

Fig. 8. Two-dimensional XRD patterns of RC fibre and non-woven RC, showing different orientation degrees.

with the relatively poor crystalline order as indicated by the width of the diffraction peaks, the maximum average crystal size was found to be of the order of only 10 nm for both materials.

3.3.6. Lipase-treated regenerated chitin fibres

Fig. 7C and D contain the XRD spectra of lipase-treated samples RC-PPL and RC-WGL, respectively. Both spectra are characterized by the co-existence of a strong and large diffuse wide-angle halo and a series of relatively sharp diffraction peaks distributed over the whole investigated angular region. Their positions are 0.34, 0.49, 0.70 and 0.95 for RC-PPL, and 0.34, 0.46, 0.70 and 0.96 for RC-WGL. The comparison of these spectra with the one in Fig. 7A clearly indicates that the exposure to lipase leads to a remarkably more ordered structure, that is presumably the result of the removal by lipases of a partially deacetylated and poorly crystalline fraction produced by NaOH during the regeneration treatment. However, the lipase treatment does not seem to have any effect on the average size of the crystal domains as analysis of the diffraction pattern did not reveal any appreciable variation of the Bragg line-widths with respect to RC.

4. Conclusions

While the chitin fibres and chitosan fibres manufactured according to the procedures of Kaifu et al. (1981); Kifune (1992); Noguchi et al. (1978); Tokura et al. (1982) maintain the spectral features of the untreated parent polymers at the XRD analysis, the DBC fibres and non-wovens resent from the chemical and technological processes to which they were submitted, i.e. the introduction (and removal) of bulky and hydrophobic butyryl groups in the high molar ratio of 2 per repeating unit, the coagulation during the spinning process, and the winding, stretching and rolling operations. Therefore, their XRD spectra contain peculiar features: in general these fibres lack several aspects of crystallinity that are present in other chitin-based fibres.

The manufacture of the non-woven materials further depresses crystallinity and orientation, possibly as a consequence of the mechanical treatments necessary for the manufacture of the non-woven, i.e. needle punching and hydroentangling.

At the ATR-FTIR analysis, the regenerated chitin fibre appears to have a lower degree of acetylation than the parent chitin, as a consequence of the alkaline treatment undergone during the hydrolysis of the butyryl ester groups. While the NaOH concentration was not very high and the temperature was kept low, one cannot exclude that the alkaline treatment could deacetylate the DBC to a certain limited extent, in consideration of the easier access of NaOH to the acetamido groups, and altered hydrogen bonding of the latter in the presence of butyryl groups.

It is therefore reasonable to report that lipases exert modest hydrolytic activity on DBC. The XRD spectra document that the removal of butyryl groups makes the long-range order normal to the fibre axis to increase; in the case of RC where butyryl groups are no longer present at any significant extent, the lipases depolymerize unspecifically a partially deacetylated and disordered polymer portion susceptible to enzymatic hydrolysis according to Muzzarelli et al. (1995).

All together, DBC does not seem particularly prone to enzymatic hydrolysis; in fact it is not hydrolysed by lysozyme according to the cited literature, and it is not degraded by lipases, as shown above. Lysozyme and lipase being the main enzymes that could release oligomers of *N*-acetylglucosamine in case of use of chitin-based textiles and non-wovens in wound management, it seems that the biochemical significance of DBC is scarce. Nevertheless, RC seems more suitable, in consideration of its better accessibility to fluids, lower hydrophobicity and scarce crystallinity. Both DBC and RC are certainly useful as supports for freeze-dried chitosans of widely recognized biochemical significance in wound healing, as well as specialty textiles.

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