CHITOSAN-BASED COATINGS FOR LIGHTLY PROCESSED FOOD

Douglas de Britto[a],*, Sérgio P. Campana-Filho[b], Odilio B. G. Assis[a]

[a]Embrapa Instrumentação Agropecuária, Rua XV de Novembro, 1452

C.P. 741, 13560-970 São Carlos – Brazil

[b]Instituto de Química de São Carlos, Universidade de São Paulo

Avenida Trabalhador São-carlense, 400 – 13560-970 São Carlos – Brazil

E-mail: dougbritto@ig.com.br, odilio@cnpdia.embrapa.br

Abstract

Some alkyl-chitosan derivatives which have improved antibacterial and anti-fungal activities, such as N,N,N-trimethylchitosan, are promising agents for food application aiming its use as coating films to protect minimally processed or post-harvested products. In this work, dimethylsulfate was used as the methylating agent of chitosan to prepare a water-soluble polymer. Also, the reaction with the adequate aldehydes allowed the introduction of alkyl group containing 4, 8 and 12 carbons in the chains of chitosan. FTIR and solid-state CP-MAS ^{13}C NMR spectroscopies were used to confirm the methylation reaction and to determine the average degree of substitution as approximately 40%. The films of chitosan and its derivatives showed similar non-linear viscoelastic behaviors though the deformation mode of chitosan films is significantly affected by the methylation process. The film of chitosan exhibited the smallest modulus of elasticity but it was the most resistant, the break down being not observed in the tested range of force. The hydrophilicity of the polymers was estimated by static contact angle measurements. The film of chitosan exhibited the higher angle (~100º) while the films of the quaternized chitosan derivatives presented a contact angle of approximately 70º, confirming the more hydrophilic character of the chitosan derivatives. The better results as protective coating in reducing fungi growth was found when using the N,N,N-trimethylchitosan.

Introduction

Due to its particular properties, chitosan is currently being used in a number of applications in the pharmaceutical[1] and food[2] industries, as wastewater separation membranes[3] and as edible coatings[4]. In particular the use of chitosan coatings to extend shelf life and improve the quality of fruits and vegetables has received considerable attention in the last few years [5-7].

However, to be useful as a protective coating the chitosan film should also exhibit adequate mechanical properties. Additionally the acid aqueous media, necessary to solubilize the chitosan can represents a serious drawback to the mechanical properties. Moreover the biocompatibility is reduced at low pH and degradation reactions easily take place on food, affecting color and flavor. To overcome these problems water-soluble chitosan derivatives have been proposed as an alternative material [8].

Alkyl derivatives of chitosan have shown improved mechanical properties and a hydrophilic character appropriated for food application [9]. To prepare such water soluble derivatives, the strategy is to introduce permanent positive charges in the polymer chains, conferring to them a

cationic polyelectrolyte feature independently of the pH of the medium[10,11]. It can be attained, e.g., by the quaternization of the nitrogen atoms of he amino groups by means of extensive methylation. The methylation of chitosan in a suspension of dimethylsulfate in the presence of sodium hydroxide has proved to be a new and efficient method [12] to prepare N,N,N-trimethylchitosan, adequate for forming protective coatings, in special for lightly processed fruits.

Materials and Methods

The starting chitosan was purchased from Polymar (CE, Brazil) and used as received. Dimethylsulfate (DMS) was purchased from Vetec (R. Janeiro, Brazil); butyraldehyde, octylaldehyde, dodecyl aldehyde and sodium cyanohydrohydride (NaCNBH$_4$) from Acros Organics and the other solvents supplied by Synth (S. Paulo, Brazil). The alkylation was carried out by reacting the chitosan with desired aldehyde dissolved in ethanol at the fixed molar ratio, as described elsewhere[9]. Thus, the aldehyde solutions employed in the alkylating reaction accounted for the following molar ratios: 2/1 butyraldehyde/chitosan, 0.5/1 octylaldehyde/chitosan and 0.1/1 dodecyl aldehyde/chitosan and the products resulting from these reactions were named as *ButChi*, *OctChi* and *DodecChi*, respectively.

For the methylation reaction 1 g of sample was suspended in 16 cm^3 of DMS and 4 cm^3 of deionized water, followed by the addition of NaOH and NaCl, according to a sequence previously established [12]. The dispersion was homogenized at room temperature. At the end of the reaction the alkyl-chitosan derivative was submitted to dialysis and the final product was precipitated upon addition of acetone.

Solid-state CP-MAS ^{13}C NMR spectroscopy (Varian Unity Inova 400) was used to characterize the derivatives, combined with techniques of proton dipolar decoupling (DD), magic angle spinning (MAS) and cross-polarization (CP). The contact time was 1 ms, the acquisition time 51.2 ms and the recycle delay 4 s. Typically, each spectrum was acquired from 2000 scans. The chemical shifts were externally referenced by setting the methyl resonance of hexamethylbenzene (HMB) to 17.3 ppm.

The films of chitosan and alkyl-chitosan derivatives were cast from its aqueous solutions. Aqueous acetic acid (1%) was used to dissolve chitosan and its alkyl derivatives while the N-quaternized derivatives were dissolved in deionized water. The polymer concentration was 10 g dm^{-3} and the films were prepared by casting on acrylic surface. FTIR (Perkin Elmer spectrometer) and DMA analysis (TA Instrument DMA 2930, tensile mode, heating rate: 5ºC mim^{-1}, amplitude: 20μm and frequency: 1 Hz) were carried out.

The hydrophilicity of the derivatives was estimated by static contact angle measurements using the sessile deionized water drop (around 5μL). For this, the polymer solution was deposited on a clean glass sheet and allowed to dry at room temperature and 40% RH gradient for 24 hrs. Images of water droplets on the film surface were recorded using a video based device and the angles were determined by FTA32 Image Software (First Ten Ångstroms). The recorded angle is the average of fives measurements in each sample. All measurements were performed under air atmosphere at room temperature.

The action of the chitosan-based coatings was evaluated by depositing it on the cut surface of apples. These apples, cv. Gala (*Malus domestica*), were first sliced into two halves and then displayed separately in two groups. The first group underwent chitosan coating by directly dipping it into the polymer solution. The excess of polymer solution was allowed to drain off and the coating was then formed by spontaneous drying at room temperature. The apples were stored at room conditions and its appearance was qualitatively verified by means of visual daily assessment.

Results and Discussion

The evidence for the introduction of alkyl substituent at the chitosan amino sites comes from FTIR spectroscopy. For alkylated chitosan (Fig. 1) a sharp increase of bands near 2840-2960 cm^{-1} is observed, which is attributed to C-H stretching of CH$_2$ and CH$_3$ groups of the alkyl substituent. Other important evidence of the alkylation is the presence of two intense bands at 1377 and 1464 cm^{-1}. The first one is associated to C-N stretching and its occurrence is in accordance to the fact that the alkyl chain was introduced at nitrogen atom. The second band, due to asymmetric angular deformation of C-H, also confirms the presence of the CH$_2$ and CH$_3$ in the alkyl-chitosan chains.
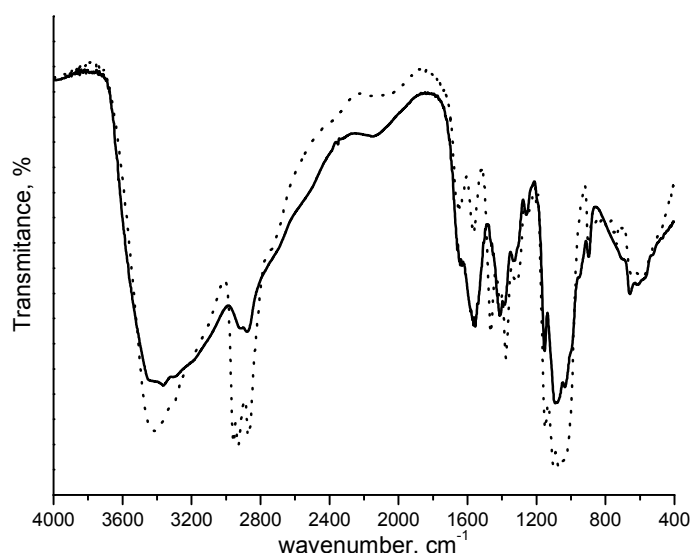

Figure 1 : FTIR spectra of chitosan (⎯⎯⎯) and alkyl-chitosan ButChi (........)

The main difference noticed in the FTIR spectra after quaternization is related to the bands at 1593 and 1464 cm^{-1}. The first, which is attributed to angular deformation of N-H bond of the amino group, is absent in the spectrum of the quaternized chitosan derivative as a consequence of the N-methylation. Similarly, the second band can be related to angular deformation of C-H and it is comparatively more intense in the quaternary derivative spectrum.
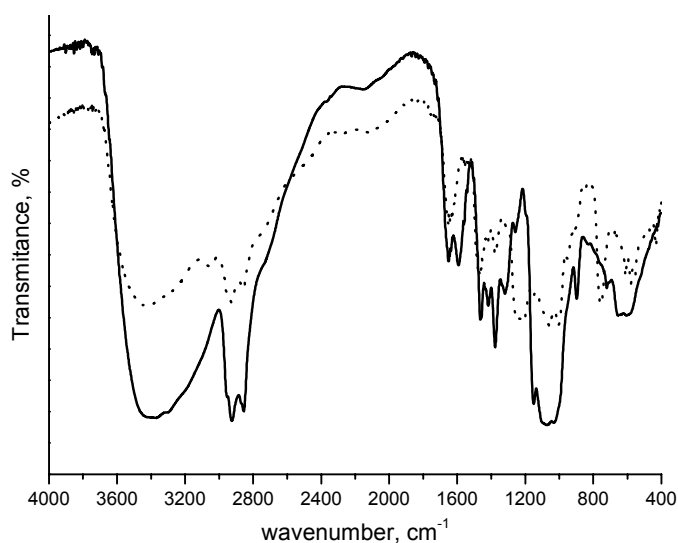

Figure 2 : FTIR spectra of alkyl-chitosan OctChi (⎯⎯⎯) and its N-quaternized derivative OctChiQt (.......).

The introduction of alkyl substituent in the chitosan chain is also confirmed by CP-MAS ^{13}C NMR spectroscopy (Figure 3) in agreement with others works[13-17]. On the other hand, for the derivative ButChi, peaks suggesting four carbons in the alkyl substituent are identified in the range 0-54 ppm. The methyl carbons of the quaternized amino sites occur at approximately 55 ppm, but its peaks overlap with additional signals. Although, some evidence of N,N-dimethylation (45.6 ppm) and O-methylation (67.9 ppm) is observed.
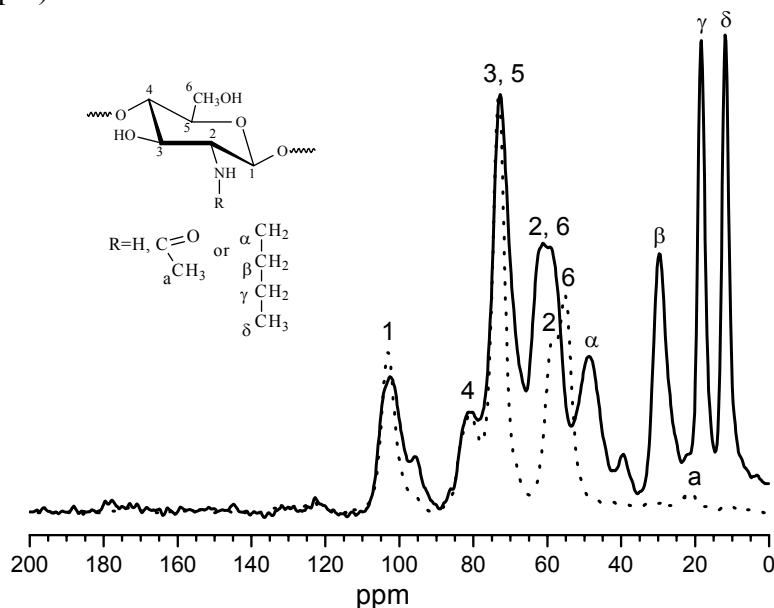

Figure 3 : CP-MAS ^{13}C NMR spectra for chitosan (……) and the alkyl-chitosan ButChi (━━).

Concerning the mechanical properties, the films of chitosan and its derivatives present similar non-linear viscoelastic behaviors as revealed by DMA but the deformation mode of chitosan films is significantly affected by the alkylation and quaternization processes. The stress-strain curves for the hydrophobic alkyl-chitosan derivatives exhibit highly elastic behavior with greater elongation while the films of the N-quaternized chitosan derivatives have a small modulus of elasticity and exhibit a typical viscous behavior. The parent chitosan exhibited the smallest modulus of elasticity, although it was the most resistant film as its breaking was not observed in the range of force employed in the mechanical evaluation. On the other hand, the alkyl-chitosan ButChi showed the highest modulus of elasticity but the film broken when elongation was near 13.5%. This results shows that the quaternization of the chitosan amino sites makes the chitosan film more fragile, reducing the modulus of elasticity and break up to 3%.

The recording contact angles (Table 1) indicate that the alkyl-chitosan derivatives do not exhibit higher angles when compared to those measured on the parent chitosan film, except for DodecQui with contact angle of ~110º. The reasons for this discrepancy are not completely clear. Considering that contact angle phenomenon is actually a very complicated subject; surface roughness, chemical heterogeneity, sorption layers, molecular orientation, swelling, and molecular weight are factors which can decisively influence the collected data. Nevertheless, for the N-quaternized chitosan derivatives it is possible to observe a decrease in the contact angle values when compared to the parent sample. In fact, the introduction of permanent positive charges in the polymer chains must increase the hydrophilic character of the derivatives.

When deposited on fruit cut surfaces, chitosan forms a high transparent film. The antifungal characteristic of chitosan was already discussed in a previous work[7]. Here the efficiency of the chitosan-based coatings to inhibit the growth of fungi was tested qualitatively by image analysis on apples. The samples covered with chitosan film (Figure 5b) had a similar behavior to that reported early, in which after 5 days the control samples showed appearance of fungi with progressive growth in the subsequent days. The best important feature achieved in the present work is related to the quaternary salt of chitosan (N,N,N-trimethylchitosan) (Figure 5c).

Table 1: Values of contact angle for chitosan, alkyl-chitosan derivatives and its N-quaternized salts.

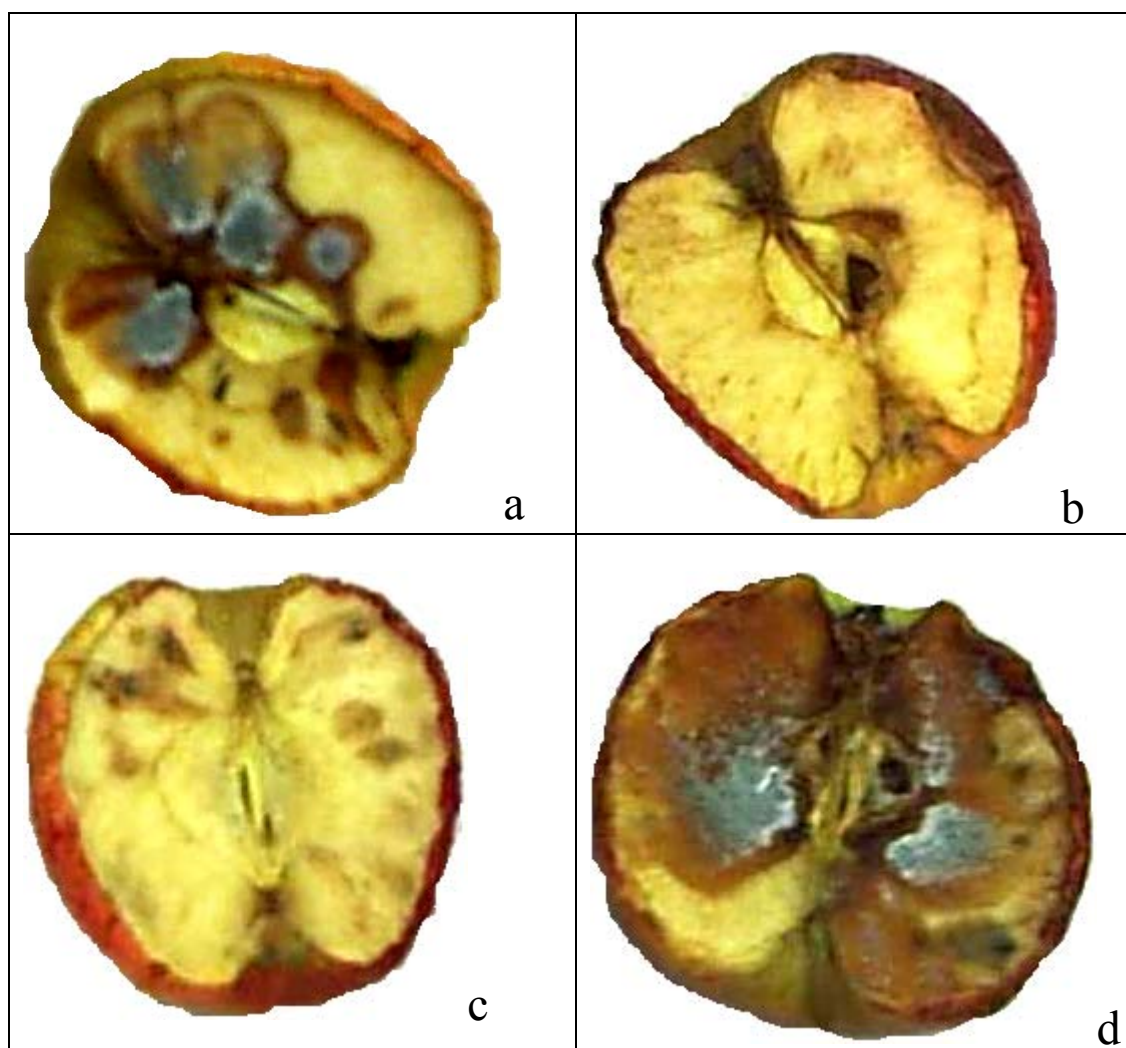| Sample	Contact Angle (º)
Chitosan	89±1.0
ChitosanQt	79±3
ButChi	74±2.1
ButChiButQt	67±1.8
DodecChi	110±1.0
DodecChiQt	76.8±0.7
OctChi	90.4±0.4
OctChiQt	96.3±0.9

Figure 4 :   Examples of cut apple surfaces a) control (uncoated sample) and coated with b) chitosan; c) quaternary salt of chitosan N,N,N-trimethylchitoson and d) quaternary salt of alkyl chitosan OctChiQt after 5 days of storage in non-controlled environment .

Indeed, the samples coated with the film of N,N,N-trimethylchitosan showed the same appearance as those coated with the chitosan film, evidencing the inhibitory character of the N-quaternized chitosan derivative. Additionally this film is deposited from a neutral solution, an important

advantage when the acid provoked spoilage of food is concerned. In fact, the low pH represents a serious drawback to the biocompatibility, affecting the color and flavor of the crop. Thus, the present result is an important improvement in this area. On the other hand, the N-quaternized salts of alkyl-chitosan do not showed a good performance against the growth of fungi. The surface of the apple coated with the OctChiQt film was the worst result (Figure 5d). This sample spoiled faster than the control sample.

References

[1] Ko, J.A.; Park, H.J.; Hwang, S.J.; Park, J.B.; Lee, J.S. **Int. J. Pharm.** 249, 165, 2002.

[2] Agulló, E.; Rodríguez, M. S.; Ramos, V.; Albertengo, L. **Macromol. Biosc.**, 3, 521, 2003.

[3] Pietrelli, L.; Xingrong, L. **Annali di Chimica**. 94, 389, 2004.

[4] Assis, O. B. G.; Pessoa, J. D. C. **Braz. J. Food Sci. Tech**., 17, 17, 2004.

[5] Worrell, D.B.; Carrington, C,M.S.; Huber, D.J. **Post. Biol. Technol**., 25, 33, 2002.

[6] Shahidi, F.; Arachchi, J.K.V.; Jeon, Y-J. **Trends in Food Scien. & Techn.**, 10, 37, 1999.

[7] Assis, B.G.O., Leoni, M.A. **Biotec. Ciência e Desenvolvimento**. 30, 33, 2003.

[8] Lazaridou, A.; Biliaderis, C.G. **Carbohyd. Polym.**, 48, 179, 2002.

[9] Desbrières, J., Martinez, C., Rinaudo, M. Internat**. J. Biolog. Macromol.**, 19, 21, 1996.

[10] Curti, E.; Britto,D.; Campana Filho, S.P. **Macromol. Bioscience**, 3, 571, 2003.

[11] Britto, D., Campana Filho, S.P. **Polym. Degrad. and Stability**, 84, 353, 2004.

[12] Britto, D.; Assis, B.G.O. **Carbohyd. Polym.**, submitted.

[13] Duate, M.L.; Ferreira, M.C.; Marvão, M.R.; Rocha, J. **Internat. J. Biolog. Macromol.**, 28, 359, 2001.

[14] Cervera, M.F.; Heinämäki, J.; Räsänen, M.; Maunu, S.L.; Karjalainen, M.; Acosta, O.M.N.; Colarte, A.I.; Iliruusi, J. **Carbohyd. Polym.**, 58, 401, 2004.

[15] Velde, K.V.; Kienkens, P. **Carbohyd. Polym.**, 58, 409, 2004.

[16] Nunthanid, J.; Laungnat-anan, M.; Sriamonsak, P.; Limmatvapirat, S.; Puttipipatkhachorn, S.; Lim, L.Y.; Khor, E.; **J. Controll. Release**, 99, 15, 2004.

[17] Holappa, J.; Nevalainen, P.; Savolainen, J.; Soininen, P.; Elomaa, M.; Safin, R.; Suvanto, S.; Pakkanen, T.; Másson, M.; Loftsson, T.; Järvinen, T. **Macromol.,** 37, 2784, 2004.