

Evaluation of Mechanical Properties and Water Vapor Permeability in Chitosan Biofilms Using Sorbitol and Glycerol

*Catarina Motta de Moura, Jaqueline Motta de Moura, Jaqueline Pozzada dos Santos, Roberta da Costa Kosinski, Guilherme Luiz Dotto, Luiz Antonio de Almeida Pinto**

Summary: Chitosan biofilms were prepared with and without plasticizer (glycerol and sorbitol). The physical and mechanical properties of chitosan biofilms with and without plasticizer were evaluated. Chitosan was obtained from shrimp wastes and characterized. The film forming solution (FFS) was obtained through chitosan dissolution and drying. The solution had its pH adjusted to 6.0 and oven dried (40 °C, 24 h) with forced air circulation. Chitosan biofilms without plasticizer showed a tensile strength about 36% higher than biofilms produced with plasticizer. On the other hand, biofilms with plasticizer presented superior values of elongation. The permeability of the water vapor and color presented significant difference ($p < 0.05$) between all biofilms. Chitosan/plasticizer biofilms showed higher values of water vapor permeability in relation to chitosan biofilms without plasticizer.

Keywords: biomaterials; biopolymers; chitosan; films; mechanical properties

Introduction

In the past ten years, a crescent increase in the researches about biofilms occurred in the scientific community. These biofilms are used as biodegradable packaging due its advantages in relation to the non-biodegradable plastic packaging.^[1] Biofilms are generally obtained using biological materials and plasticizers. They act as barrier to external elements, consequently, protecting the product and increasing its shelf-life.^[2] Biopolymers are largely employed to obtain biofilms, due its advantages, such as, biocompatibility and biodegradability. Polysaccharides, as a class of natural macromolecules, have the tendency to be extremely bioactive and are generally derived from agricultural feedstock or crustacean shell wastes. Chitosan, a deace-

tylated polysaccharide derivative of chitin, is multidimensional, such as in food and nutrition, biotechnology, material science, drugs and pharmaceuticals, agriculture and environmental protection.^[3] The plasticizers should be compatible with the biopolymer and most studied in relation to chitosan biofilms are sorbitol and glycerol. These polyols interact with the chitosan chains, increasing the molecular mobility and, consequently, the biofilms flexibility. In addition, the hydrophilic characteristics of the plasticizers lead to an increase in the hydrophilicity and water vapor permeability of the plastic biofilms.^[4] Glycerol and sorbitol can be considered suitable plasticizers to obtain biofilms with good characteristics. However, the plasticized biofilms with glycerol are highly sensitive to water and cannot be stored in conditions of high relative humidity. On the other hand, sorbitol can crystallize in conditions of low relative humidity.^[5] This work aimed to evaluate the physical (water vapor permeability, thicknesses and colors) and mechanical properties (tensile strength

School of Chemistry and Food, Federal University of Rio Grande, Eng. Alfredo Huch, 475, Rio Grande, RS, Brazil

Fax: (+55) 053 32338647;

E-mail: dqmpinto@furg.br

and elongation) of biofilms obtained from chitosan with and without plasticizers (glycerol and sorbitol).

Material and Methods

Production and Characterization of Chitosan

Chitosan was obtained from shrimp (*Penaeus brasiliensis*) wastes. Firstly, chitin was obtained by demineralization, deproteinization and deodorization steps.^[6] Chitosan paste was obtained by alkaline deacetylation of chitin (NaOH 421 g L⁻¹, at 130 ± 1 °C for 90 min), followed by purification according to Moura et al.^[7] Chitosan paste was dried in spouted bed to obtain a chitosan powder.^[8]

Chitosan powder was characterized according to molecular weight (M_w), deacetylation degree (%DD), energy dispersive X-ray spectroscopy (EDS), infra-red analysis (FT-IR) and scanning electron microscopy (SEM).

Average molecular weight of the chitosan was determined by viscosity method. Reduced viscosity was determined by Huggins equation, and converted in molecular weight through Mark–Houwink–Sakurada equation (Equation 1).^[9]

$$[\eta] = K M_w^\alpha \quad (1)$$

where, η is intrinsic viscosity (mL g⁻¹), M_w is viscosity average molecular weight (kDa), K and α are constants that depend on the solvent polymer system.

The major elements of chitosan surface were obtained by energy dispersive X-ray spectroscopy (Pioneer, S2 Ranger, GER).^[10] Chitosan powder was characterized by scanning electron microscopy, SEM (Jeol, JSM- 6060, JPN).^[10]

Chitosan characteristic bands and deacetylation degree were determined through FT-IR analysis. Chitosan powder was macerated and submitted to the spectroscopic determination in the region of the infra-red ray (Prestige 21, 210045, JPN), by technique of diffuse reflectance in potassium bromide.^[11] Deacetylation degree was

calculated according to Equation 2:^[12]

$$\%DD = 87.8 - [3(A_{C=O}/A_{-OH})] \frac{1}{n} \quad (2)$$

where, %DD is deacetylation degree (%), A_{C=O} is absorbance of C=O group, and A_{-OH} is absorbance of -OH group.

Reagents

Glycerol, sorbitol and acetic acid were obtained from Labsynth Ltda (BRA) and Vetec (BRA), respectively.

Biofilm Production

Biofilms were prepared with chitosan and plasticizers. Film forming solution of chitosan was obtained by dissolution of chitosan powder (2% w/w), which was dissolved in acetic acid solution (1% v/v) using moderate stirring (FISATOM, 752A, BRA) at room temperature for 120 min. The relations chitosan:plasticizer (chitosan (C), glycerol(G) and sorbitol (S)) were: 100:0 (C), 75:25 (C:G), 75:25(C:S), 75:12.5:12.5(C:G:S). The pH of solution was adjusted to 6.0 and measured using a pH meter (Marte, MB-10, BRA), in order to obtain a higher stability. Then the film forming solution was centrifuged (Fanem, Centrifugal Baby I 206 BL, BRA) at 5,000 × g for 15 min. 50 mL of film forming solution was added in Plexiglas plates in order to obtain the same amount of chitosan in all experiments. The biofilms were obtained by solvent evaporation in oven with air circulation at 40 °C for about 24 h. Finally, the biofilm samples were removed from plates and conditioned in desiccators at 25 ± 1 °C for at least 48 h prior to testing.

Biofilm Characterization

After the storage period, the film thickness was measured by a digital micrometer (Mitutoya Corp., MDC-25S, JPN) with 0.0010 mm of resolution. Mean thickness was calculated from ten measurements taken at different locations on biofilm samples, according to Ferreira.^[13]

Water vapor permeability (WVP) of biofilms was determined gravimetrically at

25 °C, using the ASTM standard method E96/E96M-05.^[14] Samples of each biofilm in the form of discs (diameter = 50 mm) were fixed with paraffin cell permeation of aluminium, containing anhydrous calcium chloride. These cells were placed in desiccators at 25 °C and 75% relative humidity. By increasing the mass of anhydrous calcium chloride (measured in intervals of 24 h for 7 days), it was possible to determine the water vapor transferred through the biofilm according to Equation 3.

$$\text{WVP} = \frac{w_{\text{ab}}}{t} \frac{L}{A \cdot \Delta P} \quad (3)$$

where, w_{ab} is the amount of adsorbed water (g), t is the time (days), L is the average biofilm thickness (mm), A is the area of the exposed biofilm surface (m²) and ΔP is the partial vapor pressure difference across the biofilm (Pa).

Tensile strength (TS) and elongation percentage (E) at break point were measured uniaxially by stretching the specimen in one direction using a Texture Analyzer (Stable Microsystems SMD, TA.XP2i, UK) according to the ASTM D-882-02 standard,^[15] with a 50 N load cell. Samples of biofilms were cut into 25 mm wide and 100 mm long strips. The initial grip separation and cross-head speed were set to 50 mm and 50 mm min⁻¹, respectively. The color parameters were determined using a system Minolta (Minolta Corpora-

tion, CR-400, USA), by measuring the three-dimensional color diagram (L^* , a^* and b^*). The parameters L^* (luminosity), a^* (red–green) and b^* (yellow–blue) were determined. A white disk (L_0^* ; a_0^* and b_0^*) was used like standard. The difference of color (ΔE), in the chitosan biofilm was calculated by Equation 4:^[16]

$$\Delta E = \sqrt{(\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2} \quad (4)$$

where, $\Delta L^* = L^* - L_0^*$; $\Delta a^* = a^* - a_0^*$ and $\Delta b^* = b^* - b_0^*$. The values of standard color are L_0^* , a_0^* and b_0^* , and the biofilm color are L^* , a^* and b^* .

One-way analysis of variance and Tukey multiple comparison tests were used to statistically determine significant differences ($p < 0.05$) among averages, using the software Statistic 7.0 (Statsoft, USA).

Results and Discussion

Chitosan Characterization

Chitosan powder presented average molecular weight of 150 ± 2 kDa and deacetylation degree (%DD) $85 \pm 1\%$. Chitosan with these characteristics is appropriate to biofilms production.^[7,17,18]

The EDS spectrum of chitosan is showed in Figure 1.

It was observed in Figure 1 that the major elements on the chitosan surface are

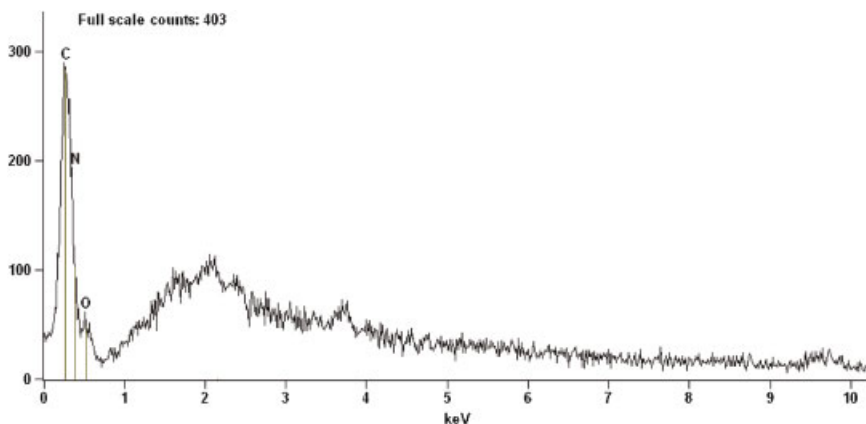


Figure 1.
EDS of chitosan powder.

C, N and O. These elements are present in chitosan functional groups, which were verified using FT-IR analysis. Figure 2 shows the FT-IR spectrum of chitosan.

SEM images of chitosan powder are shown in Figure 3. In Figure 3(a) it can be observed that chitosan powder presented porous heterogeneous surface. In addition, Figure 3(b) showed that chitosan is non-homogeneous with irregular and rough surface. These surface characteristics are important in chitosan powder applications, such as, active bio based films.^[19]

Biofilms Comparison

Table 1 shows the physical and mechanical properties of chitosan biofilms with and without plasticizer.

The Tensile strength (TS) and Elongation (E) values of chitosan biofilms without plasticizer shown in Table 1 are in the range found by the literature (40 MPa and 20% for TS and E, respectively).^[1,20] It can be observed in Table 1, the TS values of chitosan biofilms without plasticizer were higher than the TS values of chitosan biofilms with plasticizer. This occurred, probably, due to the interaction chitosan-plasticizer. In relation to the Elongation, chitosan biofilms with plasticizer presented

values about three times superior in relation to the chitosan biofilms without plasticizer. This behavior can be explained due the increase of molecular mobility, which lead to an increase in biofilms flexibility. Similar results were obtained by Epure et al.^[21]

In Table 1, it can be observed that the ΔE values of chitosan biofilms without plasticizer presented significant difference ($p < 0.05$) when compared that chitosan/glycerol and chitosan/glycerol/sorbitol biofilms. This occurred because the yellow coloration of glycerol and transparence of sorbitol. The thickness of chitosan biofilms without plasticizer was lower in relation to the thickness of chitosan biofilms with plasticizer, Table 1. This occurred due to the addition of plasticizer, which caused an increase in the amount of dry solids in the film forming solution.

A significant increase ($p < 0.05$) in water vapor permeability (WVP) was observed when plasticizers were used. Glycerol and sorbitol are polyols, however, glycerol has 3 hydroxyl groups linked with 3 carbons, and the sorbitol has 6 hydroxyl groups linked with 6 carbons. This manner, the glycerol increase the mobility of polymeric chain, leading to a decrease of intramolecular

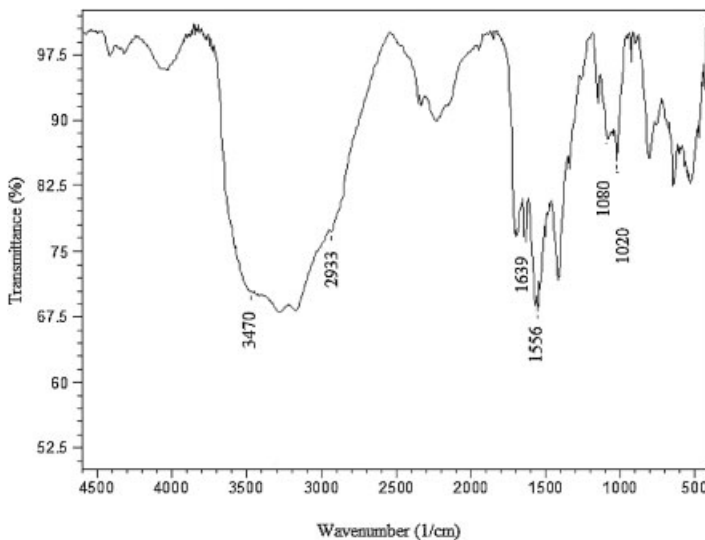


Figure 2.
FT-IR of chitosan powder.

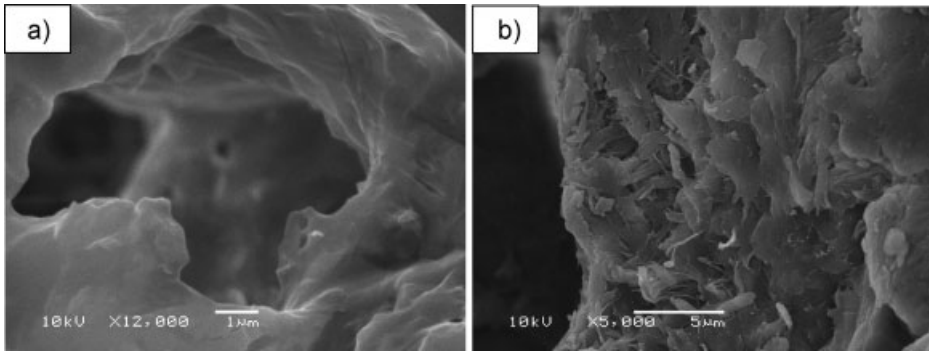


Figure 3.
SEM images of chitosan powder: (a) $\times 12,000$ and (b) $\times 5,000$.

Table 1.
Physical and mechanical properties of chitosan biofilms.

Properties	Chitosan biofilm	Chitosan biofilm		
		G ¹	S ²	G:S ³
Tensile strength (MPa)	39.2 ± 1.3^a	7.26 ± 1.6^b	14.2 ± 1.9^c	12.5 ± 1.5^c
Elongation (%)	28.3 ± 0.8^a	88.2 ± 1.3^b	89.8 ± 0.9^b	87.6 ± 1.4^b
Water vapor permeability (g mm m ² kPa ⁻¹ d ⁻¹)	3.52 ± 0.05^a	7.84 ± 0.08^b	12.3 ± 0.10^c	12.9 ± 1.20^c
Difference of color (ΔE)	8.19 ± 0.20^a	4.75 ± 0.80^c	$7.16 \pm 1.20^{a,b}$	$5.85 \pm 0.90^{b,c}$
Thickness (mm)	0.074 ± 0.004^a	0.146 ± 0.006^b	0.129 ± 0.002^c	0.121 ± 0.005^c

Mean values \pm standard error for three analyses. Equal letters in same line ($p > 0.05$). Different letters in same line ($p < 0.05$).¹⁾ Glycerol.²⁾ Sorbitol.³⁾ Glycerol:Sorbitol.

spaces and in the water binding capacity of filmogenic matrix, consequently, decreasing the WVP. On the contrary, sorbitol has a higher binding capacity with chitosan, leading to an increase in the rigidity of biofilm, and consequently, increasing its resistance. The differences between the plasticizers can be also related with the molecular weight. Plasticizers with low molecular weight, such as, glycerol, leads to the effective resistance in relation to the plasticizers with higher values of molecular weight.^[22]

Conclusion

In this work, the physical and mechanical properties of biofilms obtained from chitosan with and without plasticizers were evaluated. The chitosan biofilm without plasticizer presented percentage values of tensile strength better than the biofilms

with plasticizer. On the other hand, the biofilms with plasticizer showed the best values of elongation. The biofilms with plasticizer presented higher values of water vapor permeability in relation to the biofilms without plasticizer. The values of difference of color and thickness were also influenced by the addition of plasticizer.

Acknowledgements: The authors would like to thank CAPES (Coordination of Improvement of Higher Education Personnel) and CNPq (National Council of Scientific and Technological Development) for the financial support.

[1] P. C. Srinivasa, M. N. Ramesh, R. N. Tharanathan, *Food Hydrocolloids*. **2007**, 21, 1113.

[2] V. Coma, A. Martial Gros, S. Garreau, A. Garreau, F. Salin, A. Deschamps, *J. Food Sci.* **2002**, 67, 1162.

[3] V. V. C. Azevedo, S. A. Chaves, D. C. Bezerra, M. V. Lia Fook, A. C. F. M. Costa, *Rev. Elet. Mater. Proc.* **2007**, 2, 27.

- [4] S. Mali, M. V. E. Grossmann, M. A. García, M. M. Martino, N. E. Zaritzky, *Carbohydr. Polym.* **2004**, *56*, 129.
- [5] M. Thomazine, R. A. Carvalho, P. J. A. Sobral, *J. Food Sci.* **2005**, *70*, 172.
- [6] R. F. Weska, J. M. Moura, L. M. Batista, J. Rizzi, L. A. A. Pinto, *J. Food Eng.* **2007**, *80*, 749.
- [7] C. M. Moura, J. M. Moura, N. M. Soares, L. A. A. Pinto, *Chem. Eng. Process.* **2011**, *50*, 351.
- [8] G. L. Dotto, V. C. Souza, L. A. A. Pinto, *LWT Food Sci. Technol.* **2011**, *44*, 1786.
- [9] H. Zhang, S. H. Neau, *Biomaterials.* **2001**, *22*, 1653.
- [10] S. S. Moghaddam, M. R. A. Moghaddam, M. Arami, *J. Hazard. Mater.* **2010**, *175*, 651.
- [11] N. Sakkayawong, P. Thiravetyan, W. Nakbanpote, *J. Hazard. Mater.* **2007**, *145*, 250.
- [12] M. F. Cervera, J. Heinämäki, M. Räsänen, S. L. Maunu, M. Karjalainen, O. M. N. Costa, A. I. Colarte, J. Yliruusi, *Carbohydr. Polym.* **2004**, *58*, 401.
- [13] C. O. Ferreira, C. A. Nunes, I. Delgadillo, J. A. L. Silva, *Food Res. Int.* **2009**, *42*, 807.
- [14] ASTM. **2001**, American Society for Testing and Materials. Standard test methods for water vapor transmission of materials. Standard Designations: E96/E96M-05. In Annual book of ASTM, pp. 406–413.
- [15] ASTM. **2001**, American Society for Testing and Materials. Standard test method for tensile properties of thin plastic sheeting. Standard D882-02. In Annual book of ASTM, pp. 162–170.
- [16] M. Pereda, A. G. Ponce, N. E. Marcovich, R. A. Ruseckaite, J. F. Martucci, *Food Hydrocolloids.* **2011**, *25*, 1372.
- [17] G. L. Dotto, V. C. Souza, J. M. Moura, C. M. Moura, L. A. A. Pinto, *Drying Technol.* **2011**, *29*, 1784.
- [18] K. V. Harish Prashanth, R. N. Tharanathan, *Trends Food Sci. Technol.* **2007**, *18*, 117.
- [19] M. Aider, *LWT - Food Sci Technol.* **2010**, *43*, 837.
- [20] M. Vargas, A. Albors, A. Chiralt, C. González-Martínez, *Food Hydrocolloids.* **2009**, *23*, 536.
- [21] V. Epure, M. Griffon, E. Pollet, L. Avérous, *Carbohydr. Polym.* **2011**, *83*, 947.
- [22] R. Sothornvit, J. M. Krochta, *J. Food Eng.* **2001**, *50*, 149.