



Production and characterization of chitosan and glycerol-chitosan films

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This work presents the study of the isolation of chitin from residues of the Patagonian shrimp (Pleoticus muelleri), and its subsequent conversion to chitosan under different reaction conditions. The obtained products were characterized by evaluating their molecular weight (Mw) and degree of deacetylation (DD). In addition, chitosan- glycerol films were prepared (5, 10 and 20% w/w of plasticizer content), using a neutralization process during the unloading step. The films were characterized by infrared (FTIR) and UV-Visible spectroscopy and scanning electron microscopy (SEM). In addition, its behavior against water (contact angle and water sorption) and mechanical properties were also studied. It was observed that 72 hours of reaction time at 120 °C were necessary to obtain a chitosan sample with the desirable solubility properties from the chitin extracted from Pleoticus muelleri shells. The chitosan-glycerol films turned out to be systems of high transparency and their properties depended on the plasticizer content, obtaining homogeneous systems for concentrations up to 10% w/w. In addition, the films were found to be more hydrophilic than the reference material, with smaller contact angle and greater water absorption values, obtaining more flexible films in view of their mechanical properties.

INTRODUCTION

The field of polymeric materials has grown considerably in the last thirty years due to a significant increase in consumption. This sector is one that has been affected by the global oil crisis, because hydrocarbons derived from this industry have become scarce, and with them the raw materials for the production of traditional polymers. Therefore, there have been proposals regarding the future of raw materials and their sources to continue with the polymers production and has led to growing interest in polymers derived from renewable sources. With this in mind natural compounds have been proposed as feedstock for the preparation of polymeric materials, such as carbohydrates like starch or cellulose, or the use of natural oils [1][2]. The production of polymers from alternative renewable resources contributes to solving some of the concerns caused by the depletion of conventional petrochemical resources and adds to the areas seeking reduce environmental impact and are using green production procedures [3][4]. Among the biopolymers that combine both characteristics, are chitin and chitosan. Because of its wide distribution in nature, chitin (β (1-4) -2-acetamido-2-deoxy-D-glucose) is the second most abundant polysaccharide after cellulose. Although chitin is widely distributed in nature (fungi, algae, crustacean mollusks, insects, etc.), the exoskeletons of various types of aquatic crustaceans (shrimps, lobsters, crabs and krill) are the most accessible sources of chitin because they are waste materials from processing plants in the fishing industry. Thus, the use of this waste is an opportunity for industrial development and, at the same time, an intelligent solution for the environmental problem that they generate. However, chitin is completely insoluble in water or acid medium, which makes its use difficult in many practical applications. Deacetylation of chitin leads to a completely soluble material in an acid medium, named chitosan (β (1-4) -2-acetamido-2-deoxy-D-glucose and β (1-4) -2-amino-2-deoxy-D-glucose) whose properties depend on the reaction conditions. The presence of amino groups in the polymer chain has made chitosan one of the most versatile materials studied for some time, because of the possibility of making a wide variety of modifications from which materials with suitable properties for diverse applications are obtained. However, like other biopolymers, materials based on chitosan have inadequate mechanical properties, low thermal resistance and high permeabilities to water vapor, which has limited their use [4].

To sort out those problems, various additives can be incorporated into the chitosan (CH) films. One of the most commonly used additives are plasticizers, in particular glycerol (G), which are used in order to reduce the intermolecular attraction between polymer chains, increasing the flexibility of the film and promoting its malleability [5]. Several works in the literature have addressed the study of Chitosan-Glycerol systems [6][7][8]. From them, it is clear that the properties of these films depend on both the used chitosan (origin of the raw material, molecular weight) and the methodology used for the preparation of the films [6][9]. In this sense, other important factors are the acid used for the preparation of the CH solutions, and the application of a neutralization step during the de-casting of the films. Recently, Youssef, Abou-Yousef, El-Sayed and Kamel [10] have demonstrated the effects of the neutralization treatment on properties of CH-based nanocomposite films, in particular on mechanical properties. In general, this effect is not taken into account in most of the works where the properties of the CH based films could be affected (mechanical and water resistant behavior). This may explain in part the differences found in the literature for the variation of the properties and optimal compositions reported for these systems. Given that the processes used in the production of chitosan and chitosan-glycerol films affect the properties of both materials, this paper presents the study of the process of obtaining chitosan from Patagonian shrimp residues, as well as the morphology and properties of the chitosan

films plasticized with glycerol using a neutralization step after casting and during the unmolding process.

EXPERIMENTAL DETAILS

Materials

In this work, Patagonian shrimp shells (*Pleoticus muelleri*) from the waste of a commercial fish market were used. Sodium hydroxide (NaOH, Anedra, Argentina), acetic acid (CH₃COOH, Anedra, Argentina), hydrochloric acid (HCl, Cicarelli, Argentina) and glycerol (CH₂OHCHOHCH₂OH, Anedra, Argentina) were all of analytical grade reagents. The commercial chitosan samples used for comparison were provided by Parafarm, Argentina (CHP, Mv: 273 kDa - DD: 95.2%) and Sigma-Aldrich, U.S.A. (CHSA, Mv: 350 kDa - DD: 70.9%).

Obtaining chitosan

Chitin isolation

The Patagonian shrimp shells (*Pleoticus muelleri*) from the commercial fish market were conditioned by manual cleaning in running water to remove the remaining animal's tissue, and then washed with distilled water, dried at room temperature for 48 hours and finally at 60 °C for 24 hours. To a finely divided shells, a 0.25M HCl solution was added until reaching a mass (g): volume (ml) ratio of 1:40, and the mixture was stirred for 3 hours at room temperature in order to carry out the demineralization process. Subsequently, the dispersion was filtered and the solid obtained was washed with distilled water until neutral pH, which was verified in the wash water. The obtained solid was dried at 60 °C for 24 hours. The product obtained from the demineralization process was subjected to a deproteinization process, by treatment with a 1M NaOH aqueous solution of in a mass (g): volume (ml) ratio of 1:15 for 24 hours with constant magnetic stirring at 50 °C [11]. After that, the dispersion was filtered and the remaining solid was washed with distilled water until neutral pH of the washing water and dried at 60 °C until constant weight. The product obtained at this stage was chitin.

Deacetylation of chitin

Chitin obtained from the previous process was treated with 50% w/v NaOH solution in a mass (g): volume (ml) ratio = of 1:50 at different temperatures and reaction times (T (°C) -t (days): 65-1, 100-1, 65-3, 90-3, 100-3 and 120-3). The obtained products were isolated by filtration followed by successive washes with distilled water until neutrality of the washing water. To determine if the products obtained corresponded to chitosan, it was evaluated qualitatively verifying if they were dissolved in a 1% v/v acetic acid solution.

Preparation of the films

Films were prepared from the obtained chitosans and from commercial chitosan samples. In all cases, a 2% w/v chitosan solution was prepared in 1% v/v aqueous acetic acid solution. To obtain the films, 10 mL of 1% v/v aqueous acetic acid solution were added to 25 mL of the above solution. The solution obtained was degassed using an ultrasound bath (15 minutes, at room temperature) and cast on a glass Petri dish 10 cm in diameter. The capsules were kept for 2 hours at room temperature and then placed in an

oven at 60 °C for 24 hours. The dried films were removed from the glass dish by treatment with 1M NaOH aqueous solution (10 mL), for 2 hours. Subsequently, they were washed with distilled water and placed on a Teflon plate at room temperature for drying. In the case of films containing glycerol, the procedure indicated above was used but adding 10 mL of 1% v/v acetic acid aqueous solution containing the appropriate amount of glycerol to prepare films containing 5, 10 and 20% w/w of the additive.

Characterization

For the determination of moisture content, a mass of clean shells (m_h) was placed in an oven at 105 °C for 24 hours. When they were removed, they were placed in a desiccator in the presence of phosphorus pentoxide until reaching room temperature and weighed again (m_s). Percentual moisture content was calculated as:

$$M (\%) = [(m_h - m_s) / m_h] \times 100 \quad (1)$$

In the case of the ash content, a mass (m_0) of ground and dry shells (105 °C, 24 hours) was placed in a porcelain crucible previously tared. The capsule was placed in a muffle (550-600 °C, 12 hours). Subsequently, it was allowed to cool in a desiccator until reach room temperature and its mass was determined (m_c). The ash content was determined as:

$$\text{Ash} (\%) = (m_c / m_0) \times 100 \quad (2)$$

The molecular weight was evaluated by viscosimetry, by determination of the drain time at 25 °C of diluted solutions of chitosan in aqueous solution of 0.1M acetic acid / 0.2M NaCl. From the obtained data, the intrinsic viscosity was determined for each polymer and subsequently, based on the Mark-Houwink-Sakurada equation, the molecular weights of the chitosan samples were determined using the constants $a = 0.93$ and $k = 1.81 \times 10^{-3}$ reported previously [9] [10]. The FTIR spectra (Nicolet 380 equipment) of shells, chitin and chitosan were acquired in transmission mode using KBr pellets. The spectra of the films of CH and CH-G were acquired in the ATR mode (monoreflexion accessory, ZnSe crystal). In both cases, 64 scans were acquired, with a resolution of 4 cm^{-1} . The degree of deacetylation (DD%) of the chitosan samples obtained was determined by FTIR spectroscopy, according to the method described by Brugnerotto, Desbrières, Heux, Mazeau and Rinaudo [14], where the absorbance of the bands present at 1420 cm^{-1} (angular deformation of CH_2) and 1320 cm^{-1} (CN stretch) are used. DD% was determined as:

$$\text{DD}\% = 100 - [((A_{1320} / A_{1420}) - 0.3822) / 0.03133] \quad (3)$$

The UV-Visible spectra of the films (approximately 50 μm thick) were obtained in the wavelength range from 200 to 800 nm with a Genesys10S spectrophotometer (Thermo Scientific). The contact angle of the films was determined using a Ramé-hart goniometer at room temperature using water as a test liquid (drop volume: 10 μl). The mechanical properties (stress vs. strain) were determined on rectangular specimens following the method ASTM D638-77a (Tensile Properties of Plastic), at a speed of 0.1 mm/s on specimens of 25 mm long by 5 mm wide. The Scanning Electron Microscopy (SEM) images were obtained using a FEI - Quanta 200 microscope, applying an acceleration voltage of 15 or 20 kV. Images of the freeze fracture cross section and the surface of the films were obtained. The samples were previously sputtered with a thin gold layer. For water uptake (WS) measurements, specimens 20 mm in diameter (mass m_0) were immersed in 15 mL of distilled water at 25 °C. After immersion, the samples were periodically removed from the water, dried superficially with filter paper and immediately weighed (m_1) before being placed back in water bath. The final absorption

value was taken after 24 hours of immersion. The relative mass uptake was calculated based on the equation:

$$WS\% = [(m_t - m_0) / m_0] \times 100 \quad (4)$$

RESULTS AND DISCUSSION

Chitosan preparation

From an initial mass of 495 g of shell waste, 85 g of dry shells were obtained with 23.5% moisture and 55.5% of ash content. The mineral fraction removed was 55%, which is in accordance with the ash content. The content of material removed during the deproteinization process was approximately 12%, which is in agreement with that reported by different authors for the protein content in these systems. Thus, it is possible to affirm that by means of the procedure carried out in this work a yield of chitin between 30-35% can be obtained from clean and dry shells. Taking into account the overall process (from waste material) these percentages are drastically reduced (approximately 8%) due to the amount of material that must be removed to obtain the raw material under appropriate conditions.

The different products were characterized by FTIR spectroscopy (Figure 1A). In the spectra, it can be observed that the bands corresponding to the inorganic carbonates in the sample of shells, located at 1420 and 874 cm^{-1} (out of plane antisymmetric stretching of the CO_3^{2-} ion and to the angular deformation) [15], are lost after carrying out the demineralization process. The deproteinization is evidenced by the decrease in the intensity of the signal located at 1654 cm^{-1} ($\nu_{\text{C=O}}$) and changes in the regions of the CH stretching modes (between 2900 and 2860 cm^{-1}) and the angular deformation of the CH_2 groups (720 cm^{-1}) present in the proteins.

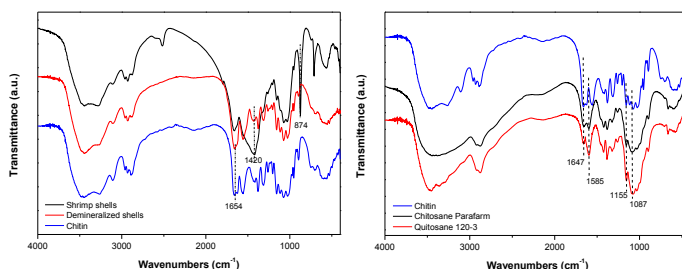


Figure 1. FTIR spectra of: **A)** clean, demineralized and deproteinized shells (chitin) and **B)** chitin, parafarm chitosan and chitosan 120-3

Table I shows the molecular weights and degree of acetylation of the different materials obtained and the commercial chitosans Parafarm and Sigma-Aldrich. The influence of time and temperature on the degree of deacetylation can be observed. It takes 3 days and a temperature higher than 120 °C to bring the chitin to a suitable degree of deacetylation so that as a result, chitosan is completely soluble in 1% v/v acetic acid solution, since at lower temperatures or shorter reaction time, obtained samples presented a partial dissolution. The intrinsic viscosity of the chitosan solutions obtained in this work (sample 120-3) and therefore the viscometry MW (M_v), is greater than the value of the commercial product from Parafarm with a similar degree of deacetylation.

The main signals observed for the CH films in the FTIR spectra (Figure 1B) were 1647 cm^{-1} ($\nu_{\text{C=O}}$, Amide I band), and 1585 cm^{-1} ($\nu_{\text{C-N}} + \delta_{\text{N-H}}$, Amide II band). In addition, the bands located in the region $1200\text{-}970\text{ cm}^{-1}$ are mainly due to the C-C and C-O stretching in the pyranose ring (centered at 1087 cm^{-1}) and to the C-O-C stretching of the glycosidic bond located at 1155 cm^{-1} , whereas the broadband located above 3000 cm^{-1} is mainly due to the superposition of the stretching vibrations of the O-H and N-H groups ($\nu_{\text{O-H}}\ 3500\text{-}3250\text{ cm}^{-1}$ and $\nu_{\text{N-H}}\ 3400\text{-}3250\text{ cm}^{-1}$, respectively) [16][17]. The obtained FTIR spectra indicate a greater similarity between the chitosan obtained in this work with the commercial chitosan Parafarm.

Table 1. Properties of commercial chitosans and samples obtained in this work

Sample	Soluble in Acetic I % ν	Insoluble fraction (%)	DD (%)		Mw _v (kDa)
			Insoluble fraction	Soluble fraction	
Parafarm	yes	-	-	95,2	273,3
Sigma- Aldrich	yes	-	-	70,9	450,1
65-1	no	100			
100-1	no	100			
65-3	no	100			
90-3	partial	90	63,9	95,6	nd
100-3	partial	10	76,0	77,9	549,8*
120-3	yes	-	-	94,5	723,5

*soluble fraction; nd: not determined

Chitosan-glycerol films

The CH-G films with thicknesses of approximately $60\ \mu\text{m}$ obtained by casting turned out to be high transparency systems. The pure chitosan film presented a high transmittance in the visible region of the spectrum (low absorbance), which is maintained with the incorporation of glycerol as a plasticizer (Figure 2B) [6]. At first sight, no imperfections were detected on the surface, and they presented a smooth texture. The films that were plasticized with glycerol exhibited greater flexibility.

Infrared (FTIR) and UV-Visible spectroscopy

The FTIR spectra of chitosan, glycerol and the samples prepared in this work can be seen in Figure 2. The contribution of glycerol in the spectra was observed by gradually increasing the intensity of the $\nu_{\text{O-H}}$ band, and in the $1100\text{-}1000\text{ cm}^{-1}$ region due to $\nu_{\text{C-O}}$, no observing other significant changes in others regions of spectra. This result confirms the effective incorporation of the plasticizer in the films.

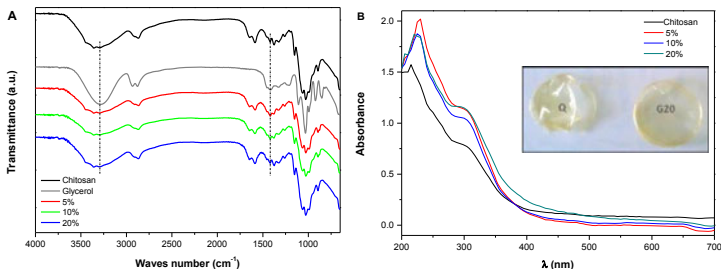


Figure 2. A) FTIR spectra of chitosan, glycerol and plasticized chitosan with 5, 10 and 20% w/w of glycerol. B) UV-visible spectra of pure chitosan and with 5, 10 and 20% w/w of glycerol. Inset in the graph are photographs of chitosan (Q) and plasticized chitosan with 20 % w/w of glycerol (G20).

Scanning electron microscopy

In Figure 3 it can be seen that the films prepared with 5 and 10% w/w of glycerol presented a homogeneous fracture surface, indicating that the addition of glycerol does not significantly affect the continuity of the polymer matrix. However, in the cross section image of the film containing 20% w/w of glycerol, two defined sections were observed: a top similar to the fracture surface of pure chitosan, and a lower front similar to the fracture surfaces of the films containing plasticizer. This suggests that for contents of 20% w/w of glycerol a phase segregated material is produced. The SEM images of the surface showed that they are smooth and uniform, without cracks or perforations, in which no morphological changes were observed after the incorporation of plasticizer [6].

Properties of the films

Table II shows the results obtained from the characterization of the films prepared in this work. In general, a slight decrease in the water contact angle was observed in films containing 5% w/w glycerol. However, the results suggest that by increasing the glycerol content this parameter becomes similar to the value observed for pure chitosan. This may be related to what is observed by SEM, where the increase in glycerol content may affect the distribution of the same in the polymer matrix leading to heterogeneous materials in terms of the distribution of the plasticizer. The chitosan showed values of tension and maximum elongation similar to those obtained by other authors for CH of similar M_v and DD [6]. The maximum elongation increased with the incorporation of glycerol, in agreement with the addition of a plasticizing material, decreasing the Young's modulus and tension at the breaking point accordingly [18]. As expected, samples containing glycerol showed a tendency to absorb a greater amount of water as the plasticizer content increase due to the hydrophilic nature of the glycerol.

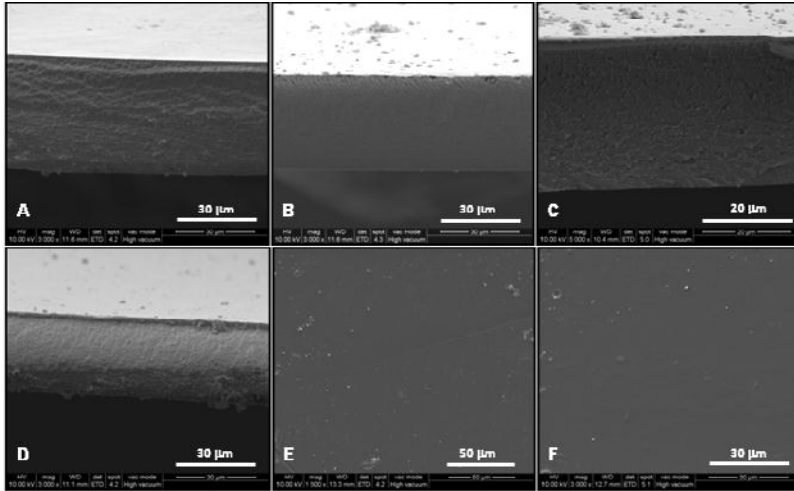


Figure 3. SEM images of chitosan film sections (A), containing glycerol: 5% w/w (B), 10% w/w (C) and 20% w/w (D) and the surfaces of the chitosan films (E) and containing 20 % w/w of glycerol (F).

Table II. Properties of the samples prepared in this work.

Sample	Water contact angle (°)	Mechanical properties			Water uptake (%)
		Stress at break (MPa)	Elongation (%)	Elastic module (MPa)	
CH	90.6	77.6	17.4	12.4	57.1
CH-G5	87.3	43.5	22.7	4.7	64.1
CH-G10	88.4	54.9	*	9.0	73.4
CH-G20	89.0	76.5	21.7	18.7	75.6

* The result of E% for the sample containing 10% w/w of glycerol is not reported because the number of valid measurements required for this determination could not be obtained experimentally.

Surprisingly, in most works non-neutralized films were studied. In this way, changes in different properties could be related to effects due to the presence of remnant organic acid which could act as plasticizer, in addition to protonate the amine groups being charged the chitosan chain, which can affect interactions between polymer matrix and the additives. Thus, in those works, is possible to find water swelling values up to 400% [19], water solubility values of the biocomposite films between 20 to 50% or an increase of WVP as CN content increase [20], and even FTIR bands assignments and interpretation based on FTIR spectrums containing remnant acid (for instance, compare Figure 4 reported in Khan A., Khan R.A., Salmieri, Le Tien, Riedl, Bouchard, Chauve, Tan, Kamal and Lacroix. Youssef, Abou-Yousef, El-Sayed and Kamel observed that the behavior of chitosan films were remarkably affected by different preparation parameter such us the type of acid for chitosan solvation, and using of concentrated alkali to assist for film removal. They concluded that the de-casting process of the films was preferred without using NaOH solution since films removal by neutralization suffer severe shrinkage (not soluble in water), while films containing organic acids presented better

mechanical properties (but water soluble) [10]. Different to this, and based on the reported information and our results, we think that the neutralization process is an important variable. In this way, the study of the optimization of the neutralization process is a central issue to obtain a balance between the properties, in addition to other variables (chitosan molecular weight and deacetylation degree, and other properties).

CONCLUSIONS

In the present work it was possible to approach the obtaining of chitosan from Patagonian shrimp residues and its use in the preparation of films. The time and the working temperature during the preparation of chitosan from chitin are fundamental variables of the procedure, affecting the degree of deacetylation and molecular weight of the final products. For the raw material used in this work, it was observed that a time of three days of reaction at a temperature of 120 °C is necessary to obtain a sample of completely soluble chitosan in acidic solution. On the other hand, the procedure employed led to a product with a higher Molecular Weight than that found for commercial chitosan samples. The obtained chitosan-glycerol films showed to be systems of high transparency, and, as expected, their properties depended on the plasticizer content. The films prepared using glycerol had a more hydrophilic characteristic than the reference material and exhibited higher water absorption as the plasticizer content increased, in accordance with the hydrophilic nature of this additive. The addition of glycerol produces more homogeneous systems when it is used in concentrations of up to 10% w/w, according to the SEM images, and this observation is in agreement with the results of mechanical properties where it was observed more flexible films for the same concentration. Thus, the properties of films composed of chitosan-glycerol depend both on the content of the additive and on the characteristics of the chitosan used for its preparation, as well as on the protocol used for its preparation.

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