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Research Article

Alkali process for chitin extraction and chitosan production from Nile tilapia (*Oreochromis niloticus*) scales

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ABSTRACT. Chitosan is a biopolymer of wide application due to its characteristics and non-toxicity, presenting antimicrobial, antitumoral and cicatrizing activities. It is currently used as emulsifier, metal's chelating, edible biofilm and fat reducer. The variation in the deacetylation degree of this polymer gives differentiated functional properties. It is mainly obtained from crustaceans, but fish scales are also a potential source of this product, despite neglected so far. The aim of this study was to develop a method for chitin extraction and deacetylation for chitosan obtaining from Nile tilapia (*Oreochromis niloticus*) scales. Characterization showed that chitosan was completely purified. The chitin infrared spectrum presented a characteristic larger band in the region of 3,500 cm^{-1}, due to axial stretching vibrations of the OH group been completely purified, which disappeared in the chitin spectrum. However, a new band aroused at 1,640 cm^{-1} due to the NH_2 deformation, which predominated over the band at 1,655 cm^{-1}, associated to the carbonyl (C=O) that tends to decrease, as the degree of deacetylation of chitosan increases. All bands observed were similar to those described in the literature. Although the yields were lower than the averages usually reported for crustaceans, they can be improved to obtain higher yields and deacetylation.

Keywords: fish residue, chitin, chitosan, infrared, deacetylation, chemical processes.

Proceso alcalino para la extracción de quitina y producción de quitosano a partir de escamas de tilapia del Nilo (*Oreochromis niloticus*)

RESUMEN. El quitosano es un biopolímero de amplia aplicación debido a sus características y no toxicidad, presentando actividad antimicrobiana, antitumoral y cicatrizante. Actualmente se utiliza como emulsionante, quelante de metales, biopelícula comestible y reductor de grasas. La variación en el grado de desacetilación de este polímero ofrece diferentes propiedades funcionales. Se obtiene principalmente de crustáceos, pero las escamas de pescado también son una fuente potencial de este producto, proceso descuidado hasta ahora. El objetivo de este estudio fue desarrollar un método para la extracción de quitina y desacetilación de quitosano a partir de escamas de tilapia del Nilo (*Oreochromis niloticus*). Esta caracterización mostró que el quitosano fue completamente purificado. El espectro infrarrojo de la quitina presentó una ancha banda característica en la región de 3,500 cm^{-1}, debido a vibraciones del estiramiento axial del grupo OH que ha sido completamente purificado, que desapareció en el espectro de la quitina. Sin embargo, se observó una nueva banda a 1,640 cm^{-1} causada por la deformación de NH_2 que predominó sobre la banda de 1,655 cm^{-1}, asociada al carbonilo (C=O) que tiende a disminuir y al aumento del grado de desacetilación del quitosano. Todas las bandas observadas fueron similares a las descritas en la literatura. Aunque los rendimientos fueron menores que los promedios reportados generalmente para crustáceos, ellos se pueden mejorar para obtener mayores rendimientos y desacetilación.

Palabras clave: residuos de pescado, quitina, quitosano, infrarrojo, desacetilación, procesos químicos.

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*), a native fish from Africa, has scales and presents vertical stripes on the caudal fin, with bluish-gray coloring (FAO, 2012). With the increasing worldwide production of tilapias (Fonseca *et al.*, 2013) summed to the low average income of tilapia fillets, around 33% (Garduño-Lugo *et*

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al., 2003), inevitably rises the amount of waste produced, including head, housing, spine, tail, viscera, skin and scale. The wastes from fish processing industries have been used for flour production, if not discarded.

The scales represent approximately 1% of total residues and do not have any nutritional value (Vidotti & Gonçalves, 2006). Their destination is usually the landfills with the other wastes, or the flour industries, to manufacture feed, despite of being a bio-unavailable residue. However, the investigation of its potential as a new raw material for chitosan production deserves to be highlighted, especially because they may be used as an alternative for people allergic to shrimp and other crustaceans.

Chitin is the exoskeletons of marine animals along with $CaCO_3$, proteins, lipids and pigments (Mathur & Narang, 1990; Percot *et al.*, 2003). It is a linear polysaccharide considered the second most abundant substance on the biomass, with the advantage of presenting a replacement rate twice that of cellulose. Chitin rarely occurs in a pure form. The complete elimination of the substances with which the chitin occurs naturally associate is not a simple task and it is sometimes very difficult to achieve a standard of purity consistent with certain applications (Campana-Filho *et al.*, 2007).

In general, the process of chitin extraction from shrimp and crab shells comprises three sequential chemical treatment steps, in order to remove associated substances, which are demineralization, deproteinization, and depigmentation/deodorization (Moura *et al.*, 2005; Battisti & Campana-Filho, 2008). Although the sequence may be changed, this seems to be the most suitable for the chitin preservation, because the binding protein/chitin preserves, to some extent, the structure of the native polysaccharide from the acid attack (Moura *et al.*, 2005). Depending on the conditions employed in these treatments, the characteristics of the obtained chitins, *e.g.*, purity and crystallinity are strongly affected (No & Meyers, 1997). Chemical synthesis of chitin is a difficult and expensive task and its production by means of biotechnological processes is not yet economically attractive (Campana-Filho *et al.*, 2007).

The deacetylation of chitin leads to obtaining chitosan, its most important derivative, whose primary structure is identical to that of chitin, except for the fact that in chitosan predominate the 2-amine-2-deoxy-D-glucopyranose units (Airoldi, 2008). The chitin deacetylation may be achieved by chemical or enzymatic processes. The chemical deacetylation of chitin can occur via homogeneous, which is carried out with alkali-chitin or heterogeneous, being the most extensively used and studied (Li *et al.*, 1997). The purpose of the deacetylation process is to remove the acetyl groups of the chitosan macromolecule chain, *i.e.*, increase the amount of free amino groups along the structure of the biomolecule. The chitosan properties depend strongly on the average degree of charge of the polymers and their distribution along the chain.

Chitosan can be utilized in a large number of industrial applications. Among its main features include biocompatibility, biodegradability, antibacterial, emulsifying and chelating properties and non-toxicity (Kimura *et al.*, 2002). The benefits provided on the chitosan consumption are innumerous, *e.g.*, regulation of digestive function, fat absorption preventing its absorption by the body, and reduction of sugar and cholesterol levels in the body (Mathur & Narang, 1990).

Chitosan reduces LDL cholesterol levels without significantly affecting HDL cholesterol levels and other essential nutrients. The indigestibility in the upper gastrointestinal tract, high viscosity, polymeric nature and low affinity for water in the lower gastrointestinal tract are factors responsible for the hypocholesterolemic effect of a fibrous diet. Chitosan meets most of these criteria and has a specific feature in relation to other fibers: *in vitro* can bind to a variety of anions, *e.g.*, bile acids and free fatty acids in solutions with low pH through ionic bonds resulting from the aminic groups (Damian *et al.*, 2005).

Besides the series of advantages, benefits and applications of chitosan, one underlines lack of information related to chitin extraction and chitosan deacetylation from fish scales. The literature reports chitin from common carp (*Cyprinus carpio*) scales (Zaku *et al.*, 2011) and chitosan from Nile tilapia (*Oreochromis niloticus*) (Uawonggul *et al.*, 2011) and *Labeo rohita* (Muslim *et al.*, 2013) scales. The aim of this work was to obtain and evaluate processes for chitin extraction and deacetylation for chitosan obtaining from Nile tilapia scales using procedures similar to those utilized for crustaceans.

MATERIALS AND METHODS

Sample preparation

The raw material utilized for chitin extraction was Nile tilapia scales. The methodology to obtain purified chitosan from raw material is described above and summarized in Figure 1.

Pretreatment

Sample were washed in water at 25°C in order to remove skin, tail, fins, and viscera residues, oven dried for 12 h at 50°C and stored in sealed plastic bag.

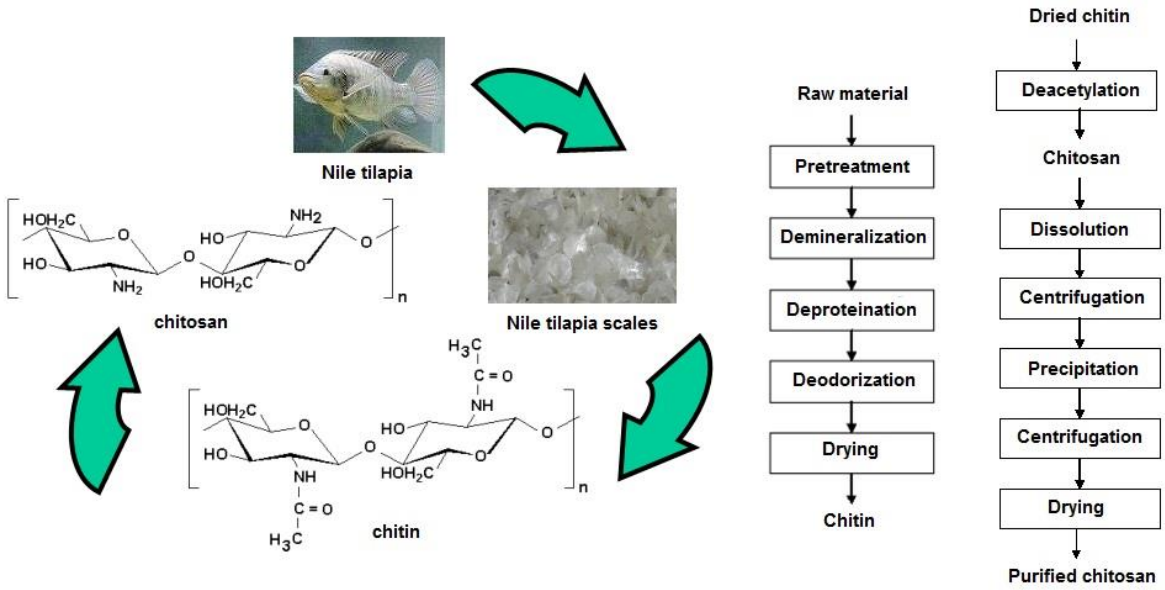

Figure 1. Scheme for purified chitosan obtaining from Nile tilapia scales (own source).

Demineralization

Demineralization was carried out by adding 1 L of 0.5 M HCl aqueous solution in 50 g of dried scales sample. The mixture was kept under stirring at 750 rpm for 2 h at 25°C. The scales were filtered, washed with water until neutrality and oven dried for 12 h at 30°C (Younes & Rinaudo, 2015).

Deproteinization

The demineralized dried material was added to 100 mL of 1% NaOH aqueous solution under stirring at 250 rpm at 50°C for 3 h. Subsequently the material was filtered, washed with water until neutrality and oven dried for 12 h at 30°C (Younes & Rinaudo, 2015).

Depigmentation and deodorizing

The deproteinized dried material was transferred to a 2 L beaker and treated with 1% NaClO aqueous solution under stirring at 500 rpm for 2 h at 25°C. Subsequently, the material was filtered and washed with water until neutrality and oven dried for 12 h at 30°C. The dried material was named crude chitin (Younes & Rinaudo, 2015).

Deacetylation

The deacetylated chitosan was obtained by suspending 10 g of crude chitin in 400 mL of 40% NaOH aqueous solution, then kept under stirring at 300 rpm for 6 h at 117°C in a glass reactor. After that, the reaction medium was filtered and the solid materials washed with water until neutrality. Then the chitosan was washed with 30 mL CH_3OH and oven dried for 12 h at 30°C (Younes & Rinaudo, 2015).

Purification

The chitosan was purified by adding 2 g of deacetylated chitosan into 750 mL of 1% $C_2H_4O_2$ aqueous solution. The suspension was stirred at 300 rpm for 20 h at 25°C. After that, the resulting solution was vacuum filtered using 0.45 μm membrane. The filtered solution was then neutralized with concentrated NH_4OH for chitosan precipitation. The precipitated chitosan solution was centrifuged at 3000 rpm for 5 min at 4°C and the supernatant removed. Chitosan was distilled water added, homogenized and centrifuged again at the same conditions. This process was repeated until the supernatant reached neutrality. The last washing was carried out with CH_3OH. The centrifuged chitosan was transferred to a Petri dish and oven dried for 12 h at 30°C (Younes & Rinaudo, 2015).

Characterization

Chitin and chitosan obtained in duplicate were characterized by absorption spectroscopy in the infrared region. The spectra in the infrared region were recorded on a FT-IR spectrophotometer 4100 (Jasco) with ATR accessory in the region from 400 to 4000 cm^{-1}. The spectra were obtained using pastilles prepared from dried chitin and chitosan samples. For that, approximately 2 mg of sample was mixed with 98 mg of KBr previously oven dried, and the mixture homogenized in

an agate mortar. The mixture was compressed in a hydraulic press to form a pellet of approximately 0.20 mm thick, which was then analyzed in triplicate (Focher *et al*., 1992).

Proximal composition

Moisture, crude protein, crude fat, crude fiber and crude ash contents were determined in triplicate according to the methods described by AOAC (2005). Moisture was determined by the oven drying method at 105°C until constant weight (method 950.46), protein by the Kjeldhal method (method 928.08) using a 6.25 factor to convert the nitrogen content into crude protein, fat by the Soxhlet method (method 960.39), and ash by using the muffle oven technique (method 920.153). Carbohydrates were calculated by difference according to Eq. 1.

$$\%CHO = 100 - (\%ASH + \%LIP + \%PRO) \quad (1)$$

where: CHO = carbohydrates; ASH: ashes; LIP: lipids; PRO: proteins. Results were expressed as% (g/100 g) by the mean and standard deviation.

RESULTS

Table 1 presents the proximate composition of Nile tilapia scales and Table 2 shows that the yield was lower than the average typically reported in studies for the extraction of the carapace of crustaceans.

It can observed the following characteristic bands in the spectrum: a large band in the region of 3,500 cm^{-1}, intense and wide, is due to axial stretching vibrations of the OH group present in chitin, which is overlapped on the N-H stretching band (Fig. 2).

Although the spectra in the infrared regions of chitin and chitosan have certain similarities, some differences can be observed, attributed to different rates of acetamide groups, mainly in the regions corresponding to the following wave number ranges of 3,700-3,000 cm^{-1} and 1,800-1,500 cm^{-1} (Fig. 3).

DISCUSSION

The protein content of scales resembles to that from soft-shell crab shell and is lower than that found in shrimp shell, with ash content much higher than those obtained for both of them (Moura *et al.*, 2005). From the proximate composition analysis it is possible to note the importance of the treatments of demineralization and deproteinization due the high contents of ash (mainly $CaCO_3$) and proteins, respectively, found in scales.

Table 2. Mean mass variation and yield extraction during the transformation of chitin into purified chitosan.

Material	Mass variation (g)	Yield (%)
Scale	50 ± 3.65	-
Chitin	10.31 ± 0.59	20
Chitosan	2.50 ± 0.19	24
Purified chitosan	0.78 ± 0.11	39

Most of the researches aiming chitosan obtaining from crustacean use ground and sieved raw materials (Basttiti & Campana-Filho, 2008). The use of the whole material decreases the contact surface with the reaction medium. Nevertheless, chitin was transformed into chitosan. However, the use of the full material may have reduced the deacetylation potential of the polymeric chain and the transformation of chitin into chitosan of higher degree of deacetylation and aggregate value. Concerning the yield, it is believed that the extraction process can be improved to obtain higher yields and deacetylation.

In a characteristic spectrum previously reported, it was found a greater number of bands (Moura *et al*., 2005), which were formed because the material has not been completely purified.

The considerably strong bands, observed between 1,700 cm^{-1} and 1,300 cm^{-1}, are well typical. The band at 1,655 cm^{-1} is attributed to the axial strain of C=O present in chitin, denominate amide I. The band at 1,581 cm^{-1} corresponds to the mixture of two vibrational modes, NH in the plane and the C-H stretching, which is called amide II. In addition, polysaccharides bands in the region between 890 and 1,150 cm^{-1} were observed in all the samples investigated (Fig. 2). All bands observed are similar to those described in the literature.

For chitosan samples is visible the disappearance of the shoulder at 3,432 cm^{-1} present in the chitin spectrum from Figure 2. The arising of a new band at 1,640 cm^{-1}

Table 1. Proximal composition of Nile tilapia scales (%) dry weight basis. *In dry basis.

Sample	Moisture	Lipid*	Protein*	Ash*	Carbohydrate*
Scale	13.08 ± 0.004	0.34 ± 0.12	54.25 ± 0.72	36.06 ± 0.67	9.21

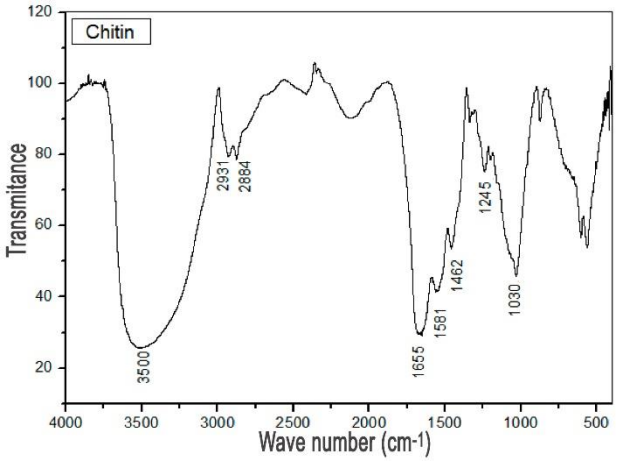

Figure 2. Infrared spectrum (FT-IR) of chitin obtained from Nile tilapia (*Oreochromis niloticus*) scales.

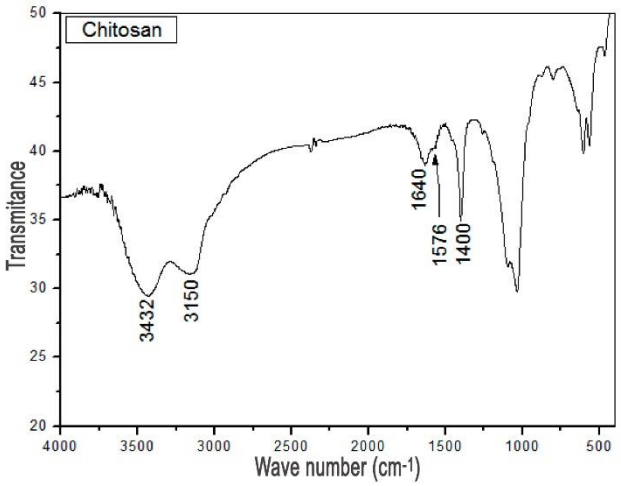

Figure 3. Infrared spectrum (FT-IR) of chitosan obtained from Nile tilapia (*Oreochromis niloticus*) scales.

and the vanishing of the band at 1,576 cm^{-1} are due to the NH_2 deformation, which predominates over the band at 1,655 cm^{-1}. This latter band is associated to the carbonyl (C=O) that tends to decrease, as the degree of deacetylation of chitosan increases. The disappearance of the two bands between the regions 3,432 and 3,150 cm^{-1}, as already mentioned, is related to deacetylation of the group $NHCOCH_3$, transforming the amide into primary amine. There was certain difficulty in characterizing the N-H band, which occurs in the same region of the C=O. The amine band in chitosan at 1,550 cm^{-1} should be pronounced. Thus, it may have suffered displacement and shortening (Fig. 3).

CONCLUSIONS

Although chitin is the second most abundant compound in nature, its obtaining cannot be considered easy, because there are few known methods for its extraction and subsequent deacetylation, to form chitosan. In this work we applied the methods of demineralization, deproteinization, deodorization, purification and deacetylation to Nile tilapia scales. The yields were lower compared to results obtained in the extraction from crustacean, but it is foreseeable that adaptation of the method to scales can bring improvements in both yield and quality of the final chitosan, although the spectra obtained for chitosan has shown that the product is apparently quite pure.

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