

EXTRACTION AND CHARACTERIZATION OF BIOPOLYMERS FROM EXOSKELETON RESIDUES OF THE AMAZON CRAB *DILOCARCINUS PAGEI*

EXTRAÇÃO E CARACTERIZAÇÃO DE BIOPOLÍMEROS DE RESÍDUOS DO EXOSQUELETO DE CARANGUEJO AMAZÔNICO *DILOCARCINUS PAGEI*

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ABSTRACT

Chitin is the second most abundant biopolymer worldwide and is found in a large variety of animals. Besides shrimps, other species possess significant chitin contents in their external non-edible fraction, thus allowing them to be also economically viable sources of this macromolecule. According to mass-loss evaluation of crab residues, 78.4% of the mass is comprised of CaCO_3 and 21.6% associated to the organic phase. The chitin content found was 8.0% of the residue's initial mass and after the deacetylation step, the average chitosan yield was 5.0% of the initial residue mass. The thermal decomposition profiles of obtained chitin and chitosan samples were characteristic of biopolymers, exhibiting non-oxidative (190–360°C) and oxidative (340–670°C) events of mass loss. Vibrational spectroscopic analysis showed that the degrees of deacetylation of the obtained chitosan samples were time-dependent and between 68.4 and 81.9%.

Keywords: Crustacea; Trichodactylidae; chitosan; chitin; degree of deacetylation.

RESUMO

Quitina é o segundo biopolímero mais abundante no mundo e é encontrada em ampla gama de animais. Além dos camarões, outras espécies possuem conteúdo significativo de quitina em suas frações externas não comestíveis e também podem ser considerados como fontes economicamente viáveis. A avaliação de perda de massa dos resíduos do caranguejo revela que 78,4% da massa é composta de CaCO_3 e 21,6% associam-se à fase orgânica. O total de quitina encontrado foi de 8% da massa inicial de resíduo, e após a desacetilação o rendimento foi de 5% da massa inicial. Os perfis de decomposição térmica das amostras poliméricas obtidas apresentaram características de biopolímeros, exibindo eventos não oxidativos (190–360°C) e oxidativos (340–670°C) de perda de massa. Os resultados da espectroscopia vibracional revelam que os graus de desacetilação obtidos para amostras de quitosana dependeram do tempo de reação e estiveram entre 68,4 e 81,9%.

Palavras-chave: Crustacea; Trichodactylidae; quitosana; quitina; grau de desacetilação.

INTRODUCTION

The freshwater crab *Dilocarcinus pagei* Stimpson, 1861, is a species abundantly found in South America, especially in Amazon (MAGALHÃES *et al.*, 2016) and, despite the wide availability of this species in the region, it has not been commercially explored on a large scale. This crab is marketed in the Pantanal region as live bait, promoting a local income for the riverside communities, and it is ruled by the state laws (MATO GROSSO DO SUL, 2011a; 2011b). However, it should be considered an important source of alternative income in the future, since the species has a promising potential to be explored, especially in the food industry as a flavoring agent (COSTA, 2015).

In general, the crab's meat is the most economically important part of this animal, particularly when considering its applications in the food segment (YEO *et al.*, 2008; SILVA *et al.*, 2018). However, the edible content of a large number of crab species is considerably smaller than the non-edible parts, such as the exoskeleton, which is often discarded. Although from the environmental point of view there are no severe impacts associated with their disposal, the residues from the meat processing of crabs can be further processed in order to aggregate value to a wasted resource. In this sense, traditionally non-edible parts have been used for elaborating flavoring products (MATOS, 2005) and obtaining chitin and chitosan (YOUNES; RINAUDO, 2015; KUMARI *et al.*, 2017).

Chitin, after cellulose, is the most abundant biopolymer found in biomass (RINAUDO, 2006). Conventionally, chitin is produced by a range of marine animals, mainly crabs and shrimps from food production (YOUNES; RINAUDO, 2015) but is also found in insects (KAYA *et al.*, 2015), fungi (WU *et al.*, 2005), and algae (CAMPANA-FILHO *et al.*, 2007). In crustaceans, chitin is associated with the other

constituents of the exoskeleton as inorganic materials, mainly calcium carbonate (CaCO₃), and organic materials such as proteins, lipids and pigments (CAMPANA-FILHO *et al.*, 2007; KUMARI *et al.*, 2017). The typical isolation of this biopolymer is achieved through chemical processing by employing acidic and alkaline solutions to remove the inorganic content and non-polymeric organic species. Subsequently, chitin is converted into another polymer of superior commercial interest through another chemical process called *deacetylation*, in which chitosan is obtained (YEN; YANG; MAU, 2009; LIU *et al.*, 2017; SAHARIAH; MÁSSON, 2017). Chitin and its chitosan derivative have peculiar characteristics and properties (biocompatibility, biodegradability, non-toxicity and biological activities), which yields multiple applications in the pharmaceutical, cosmetic, dermatological, biomaterial and agricultural industries (RINAUDO, 2006; LARANJEIRA; FÁVERE, 2009; MENDES *et al.*, 2011; CHIAPPISI; GRADZIELSKI, 2015; HAMED; ÖZOGUL; REGENSTEIN, 2016).

Most of the studies focused on extracting and obtaining chitin and chitosan from natural resources have employed marine crustaceans (AL-SAGHEER *et al.*, 2009; VÁZQUEZ *et al.*, 2013; BARON *et al.*, 2017), and few studies reported in the literature using freshwater crab species (BOLAT *et al.*, 2010), especially from the Amazon region. According to Costa (2015), the meat yield of the *Dilocarcinus pagei* crab is around 12% and the body's remains are mainly composed of exoskeleton, which is considered a waste of meat processing. Therefore, the present study shows the chemical processing of external residues of *D. pagei* crab and the physicochemical characterization of the isolated intermediate materials and chitin and chitosan samples.

MATERIALS AND METHOD

Chitin extraction and chitosan production were previously optimized and followed the steps of pre-treat-

Pre-treatment

The exoskeletons of crabs used in this study were supplied by the Zoology Laboratory from Exact Sciences and Technology Institute (ICET) of the Federal University of Amazonas (UFAM). Around 700 g of crab residues were washed manually and later oven

dried at 70°C for 5 hours. Then, the dried material was mechanically ground in a domestic blender and later passed through a 120 mesh sieve to discard coarse particles. All of the following steps were previously optimized.

Demineralization of crab residues

The step of removing the mineral content was performed using 0.50 mol L⁻¹ HCl (Kinetic) solution for 30 minutes. A ratio of 1:40 (residue mass (g)/acid solution (mL)), was employed and the materials were kept under constant mechanical stirring at room temperature after contact.

Deproteinization of the organic remnant

Demineralized material was added to a 1.0 mol L⁻¹ NaOH (Cinética) solution and the experiments were carried out using a 1:30 (m/v) ratio, with a reaction time of 24 hours, employing continuous mechanical stirring and constant temperature of 70°C after mixing

Chitin depigmentation

The deproteinized material was added to a 0.14 mol L⁻¹ NaClO (Brilux) solution using a 1:25 (m/v) ratio, and the mixture was stirred mechanically for 8 hours at 40°C after contact. Subsequently, the mixture was filtered and the solid obtained was washed with distilled water un-

Subsequently, the mixture was filtered and the solid obtained was washed with distilled water until near-neutral pH values were obtained. Thereafter, the material obtained was oven dried at 70°C for 5 hours, and the resulting solid was labeled DM1.

the materials. Later, the mixture was filtered and the solid obtained was washed with distilled water up to near-neutral pH values. Thereafter, the material was oven dried at 70°C for 5 hours, and the resulting solid labeled DP2.

til neutrality was achieved and to the point where the presence of chloride anions were no longer detected after mixing with dilute silver nitrate (Labimpex) solution. Then, the chitin obtained was oven dried at 40°C for 12 hours, and the resulting solid labeled QT3.

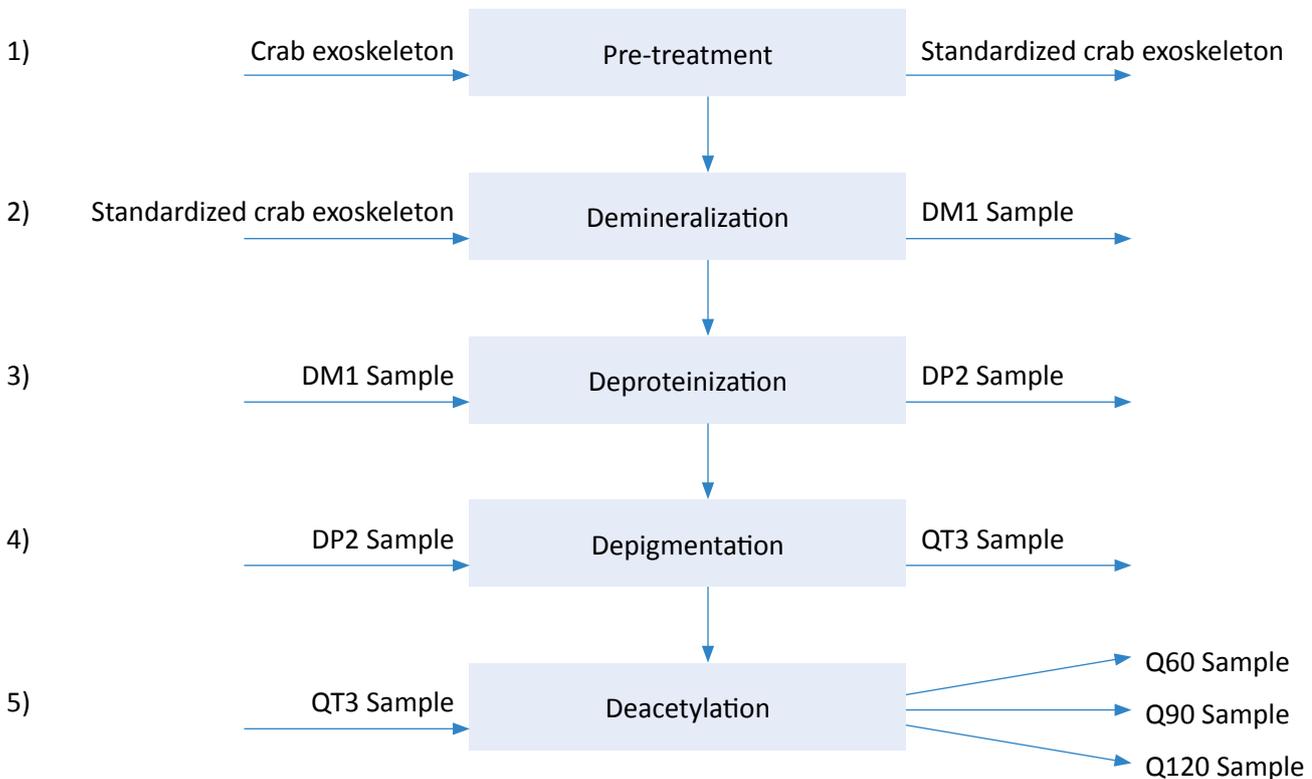


Figure 1 – Flowchart of chitin extraction and chitosan production.

Chitin deacetylation

Chitin deacetylation was carried out using a 10 mol L⁻¹ NaOH solution at 105°C under constant mechanical stirring and reaction times of 60, 90 and 120 minutes, with a ratio of 1:40 (m/v) in all cases. After completing the reaction, the obtained solid phase was filtered and

Statistical analysis

Each step of the experimental procedure was performed in triplicate. The yield of the individual steps at the chitin production process was calculated using the initial mass of ground crab residues processed and the amount of chitin obtained. The chitosan yield was obtained from determining the final mass

Characterization

The obtained materials were analyzed by the X-ray diffraction (XRD) technique in a Rigaku diffractometer, model Miniflex, using 30 kV, 15 mA, variable slits and Ni filter. The step used was 0.03° s⁻¹ and the range analyzed (2θ) was 1.5 to 70°. The powdered samples were placed in glass sample holders to record their XRD patterns.

The thermogravimetric analysis (TGA) was recorded on a Netzsch TGA equipment, model STA 409 PC Luxx coupled to a QMS 403 C Aeolos mass spectrometer employing alumina crucible, synthetic airflow (50 mL min⁻¹) and temperature range from 30 to 1,000°C, with a heating rate of 10°C min⁻¹ and using a mass of 15 mg per sample.

The Fourier Transform Infrared (FTIR) spectra were recorded in a Bomem-Michelson FTIR spectrometer, model MB-102, in the 4,000 to 400 cm⁻¹ region, with accumulation of 64 spectra for each sample. Spectra were obtained using pellets prepared from chitin and chitosan samples dried for 12 hours at 40°C and

washed with distilled water until neutrality. The processing was finished after drying the material in an oven at 40°C for 6 hours, and the resulting solids were labeled Q60, Q90 and Q120, respectively, according to their reaction time.

in relation to The initial mass of chitin used in the deacetylation step. The data were statistically analyzed using Origin software (version 8.0). The chitosan mass data were submitted to analysis of variance (ANOVA), one-way, with significance level of $p < 0.05$.

KBr (Merck). About 2mg of sample were mixed with 98mg of previously dried KBr and the mixture homogenized in agate mortar. The blend was pressed into a hydraulic press to form a disk approximately 0.20 cm thick and then analyzed. Through the ratio between the amide band I absorbance at 1655 cm⁻¹ and the hydroxyl band at 3450 cm⁻¹, the degree of deacetylation (DD) of the obtained chitosan samples was calculated, as shown in Equation 1 (CANELLA; GARCIA, 2001):

$$DD = (A_{1655} / A_{3450}) \cdot 100 / 1.33 \quad (1)$$

In which:

A₁₆₅₅ = absorbance at 1,655 cm⁻¹;

A₃₄₅₀ = absorbance at 3,450 cm⁻¹;

1.33 = constant representing A₁₆₅₅ / A₃₄₅₀ ratio for completely N-acetylated chitin samples.

RESULTS AND DISCUSSION

After the crab residues' reaction with the 0.50 mol L⁻¹ HCl solution, it was observed that the DM1 sample had a mass reduction of 71.3% of inorganic content extracted from the initial mass and about 28.7% average yield of demineralized material. Hence, it can be observed that the exoskeleton of *D. pagei* crab has a high mineral content in its structural composition. Depending on the species of crustaceans and the local they are found,

the structural constitution of the exoskeleton can present different amounts of inorganic (mainly CaCO₃) and organic compounds (lipids, proteins and chitin). Percot, Viton and Domard (2003) reported the optimization of the shrimp exoskeleton demineralization process using 0.25 mol L⁻¹ HCl solution and a reaction time of 15 minutes. However, these conditions were not optimized for the *D. pagei* crab since there is a greater

amount of CaCO_3 in its exoskeleton, requiring a higher acid content to remove the inorganic fraction. Owing to this greater amount of carbonate, it is necessary not only to increase the amount of acid but also the reaction time, since not all CaCO_3 reacts readily with HCl due to the high compaction of its biological structure.

By doubling the acid concentration and the reaction time, a mass reduction of 71.3% of the initial crab residue was observed, and this result shows that the exoskeleton of this studied species has a high mineral content. A similar result was reported by Oliveira and Nunes (2011), who found in the residues of the mangrove crab *Ucides cordatus* 61.2% of inorganic content from the initial mass. The same authors also affirm that the high mineral content is one of the factors that difficult the demineralization process needed in order to obtain chitin. The mineral percentage found in the present study is relatively higher in relation to the results reported in the literature for the exoskeleton of other species of crustaceans. Younes *et al.* (2014) found a percentage of 35.3% of inorganic constituents for *Metapenaeus monoceros* prawn. It should be emphasized that the different values of mineral content reported in other studies may be directly related to the species, genera and seasonality of each one, which may justify the different experimental conditions employed in other studies (AL-SAGHEER *et al.*, 2009; ARBIA *et al.*, 2013).

On the other hand, alkali treatment of the demineralized material (DM1), the DP2 sample showed a mass reduction of 51.3% in relation to the initial amount of demineralized material, presenting an average yield of 48.7%. Considering the initial mass of crab residues, the results reveal that, initially, the *D. pagei* crab's residues contained 11.0% of lipids and proteins in their composition and 10.6% of deproteinized organic material, consisting essentially of pigments and polymeric material.

The percentage of lipids and proteins found in this study was also similar to those obtained by Oliveira and Nunes (2011) in the exoskeleton of the *U. cordatus* crab (13.2%). However, the percentage of protein and lipids found in this study is significantly lower when compared to those reported in the literature for different prawn species. Charoenvuttitham, Shi and Mittal (2006) and Benhabiles *et al.* (2012) found that the exoskeletons prawn of the *Penaes monodon* and

Parapenaeus longirostris contained about 47.4 and 40.6%, respectively, of both biomolecules in their structural composition.

However, the contact of the demineralized sample with a 1.0 mol L^{-1} NaOH solution during the deproteinization stage was not enough to remove the pigments present in the structure of the crab's exoskeleton as evidenced by the dark brown color of the deproteinized material (image not shown). Commonly, the exoskeleton of crabs possesses a wide range of organic compounds, especially carotenoid pigments, such as astaxanthin (OGAWA *et al.*, 2007). Several procedures for removal of the non-polymeric organic content involving mainly oxidizing compounds, such as potassium permanganate and sodium hypochlorite (LIU *et al.*, 2012; KAYA; BARAN; KARAARSLAN, 2015) are described. It was observed that after the contact of the DP2 sample with NaClO solution, the resulting solid (QT3 sample) exhibited a light brown coloration (image not shown), evidencing the reduction of this organic fraction in the final material.

After the removal of the non-polymeric organic content, the sample is composed essentially of chitin. For this step, a final mass attributed to chitin of $3.77 \pm 0.05 \text{ g}$ was obtained starting from 5.00 g of deproteinized material for each replicate. Therefore, the mass of the DP2 sample showed a mass reduction of 22.8% when converted to the QT3 sample, and the mass lost is associated to the pigment decomposition and possibly partial fragmentation of chitin. Assuming that this reduction of mass is due exclusively to the removal of the pigments, the total percentage of pigments found in the crab residue was 2.6%. The total amount of chitin present in the exoskeleton of the crustaceans and the yield of the conversion process of chitin to chitosan varies depending on the species analyzed and the route of production employed (ARBIA *et al.*, 2013; KAYA *et al.*, 2014). Table 1 shows the percentages of chitin and chitosan found in this study and other reports for different species of crustaceans.

In the conversion process of chitin to chitosan, strong alkali concentration and temperature above 100°C are generally required. Since the reaction time determines the degree of deacetylation (DD), it was possible to obtain chitosan with different characteristics. From the statistical point of view, the data shown in Table 2 indicated that there was no difference ($F = 3.52$; $df = 2$;

$p = 0.0975$) between the mass values obtained using different lengths of reaction, thus not presenting a correlation between yield and time of reaction. Once again from this data, it was found that the chitosan produced represents 5.0% of the initial mass of crab residues. Therefore, the total chitin and chitosan content found for *D. pagei* crab has values close to those found for others crustaceans (HENNIG, 2009; BOLAT *et al.*, 2010; BILGIN; FIDANBAŞ, 2011; SANTOS; CIRILO; NUNES, 2011; BÖLGEN *et al.*, 2016; KAYA *et al.*, 2016).

For the chitosan samples obtained, no statistical differences were observed in their final masses, which were around 62.0% of the initial chitin mass employed in the deacetylation step. Hence, 8.0% of polymeric material in the form of chitin and 5.0% in the form of chitosan were obtained from the initial mass of *D. pagei* residues.

The XRD patterns of the solids from crab residues after each step (demineralization, deproteinization, depigmentation and deacetylation) performed under opti-

Table 1 – Percentages of chitin and chitosan found in the exoskeleton of different species of crustaceans.

Crustacean	Species	Chitin Content (%)	Obtained Chitosan (%)	Authors
Freshwater Crab	<i>Dilocarcinus pagei</i>	8.0	5.0	Present study
Freshwater Crab	<i>Potamon potamios</i>	6.83	4.65	BOLAT <i>et al.</i> , 2010
Freshwater Crab	<i>Potamon potamios</i>	7.80	5.86	BILGIN; FIDANBAŞ, 2011
Mangrove Crab	<i>Sesarma plicatum</i>	18.46	7.64*	SAKTHIVEL; VIJAYAKUMAR; ANANDAN, 2015
Blue Crab	<i>Callinectes sapidus</i>	12.1	9.20*	KAYA <i>et al.</i> , 2016
Blue Crab	<i>Callinectes sapidus</i>	11.73	9.12*	BÖLGEN <i>et al.</i> , 2016
Prawn	<i>Penaeus brasiliensis</i>	5.3	2.5	HENNIG, 2009
Shrimp	<i>Macrobachium jelskii</i>	5.9	5.06	SANTOS; CIRILO; NUNES, 2011

*Determined values from the data reported of the respective studies.

Table 2 – Chitosan yields obtained at different reaction times.

Chitin (g)	[NaOH] (mol L ⁻¹)	Time (min)	Chitosan (g)	Mass (g)	Sample name
1,000	10	60	0.657	0.64 ± 0.01	Q60
			0.620		
			0.635		
1,000	10	90	0.626	0.62 ± 0.01	Q90
			0.625		
			0.613		
1,000	10	120	0.604	0.61 ± 0.01	Q120
			0.616		
			0.614		

mized conditions are shown in Figure 2. This technique allows the visualization of the organizational level of the samples' constituents, offering a way to evaluate the impact of the process of inorganic and non-polymeric organic fractions removal in the final composition of the obtained materials. For the DM1 sample, diffraction peaks were found at (2θ) 9.3, 12.5, 19.3, 26.5 and 35.0°, which are characteristic of α -chitin phase (CAMPANA-FILHO *et al.*, 2007; AL-SAGHEER *et al.*, 2009).

For the deproteinized sample, DP2 diffraction peaks at (2θ) 9.3, 12.7, 19.3, 23.5, 26.5, 34.8 and 38.9° were observed. Comparing the XRD patterns of DM1 and DP2 samples, it can be observed that there were sig-

nificant differences between them, mainly related to the peaks' intensity and width. The differences observed between the diffraction patterns of deproteinized and demineralized (DM1) materials (DP2) can be explained by the fact that in the DM1 material, after the demineralization process, there was still a considerable amount of non-polymeric organic compounds that significantly interfere in the interaction among the chitin chains, preventing the appearance of several diffraction peaks, characteristic of the α -chitin phase. As a result, the presence of broader and less intense X-ray diffraction signals was observed for DM1 in comparison to the DP2 sample.

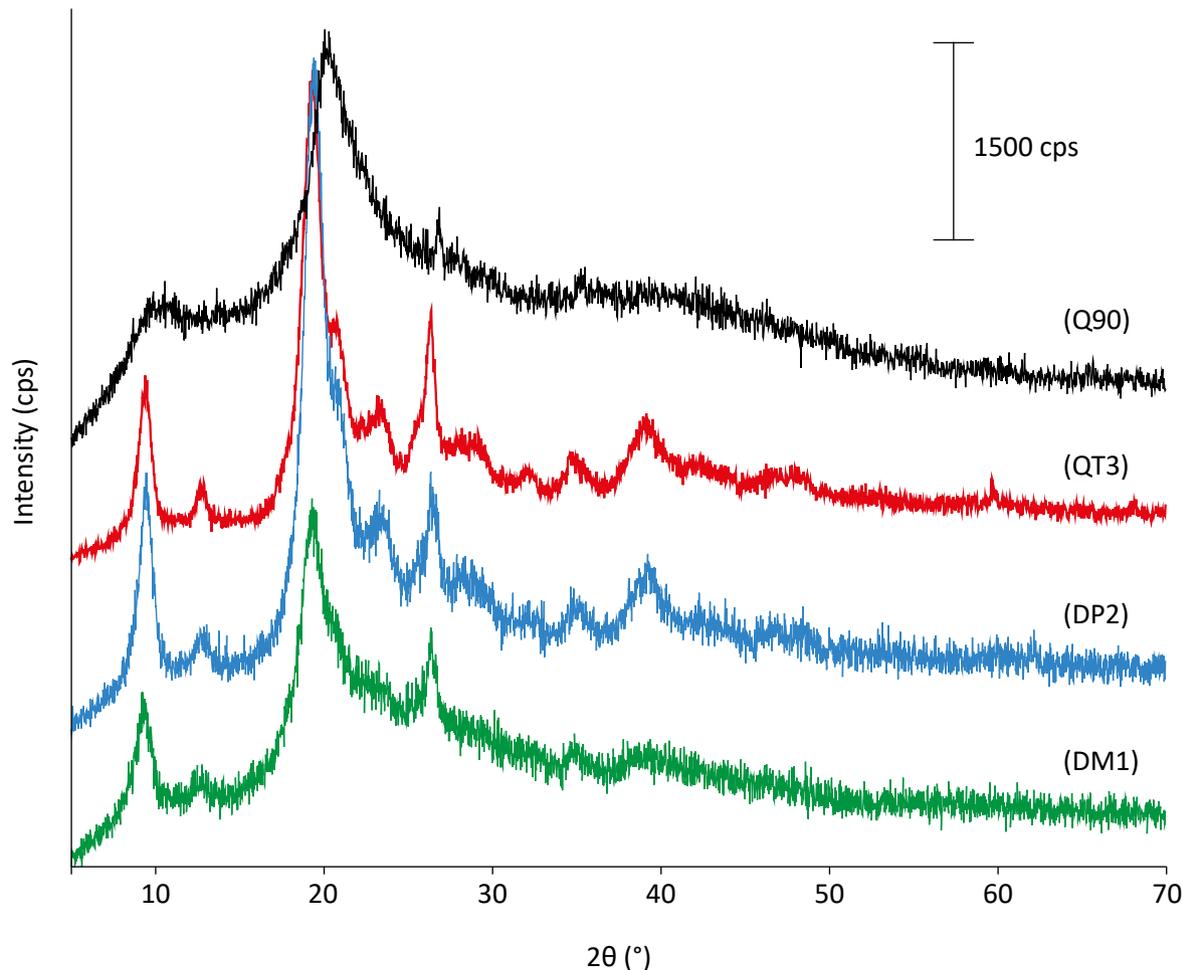


Figure 2 – X-ray diffraction (XRD) patterns of the materials obtained from crab residues: demineralized sample using 0.50 mol L⁻¹ solution of HCl for 30 min (DM1 - green); deproteinized sample using 1.0 mol L⁻¹ NaOH solution for 24 h (DP2 - blue); depigmented sample using 0.14 mol L⁻¹ NaClO solution for 8 h (QT3 - red) and deacetylated sample using 10 mol L⁻¹ NaOH solution for 90 minutes (Q90 - black).

For the QT3 sample, it was observed the presence of intense reflections at (2θ) 9.32 and 19.38° and less intense peaks at (2θ) 12.70, 23.45 and 26.37°. This is due to the removal of the pigment content in the deproteinized material alters positively on the extent of the interactions among the chitin chains, leading to greater crystallinity of the remaining polymer phase. The chitosan obtained through deacetylation process for 90 min (Q90) presented two peaks at (2θ) 10.12 and 20.11°, which were fewer, broader, and less intense than those obtained for α -chitin (QT3), indicating a lower level of crystallinity after the chemical treatment to produce chitosan. Chitosan, commonly a partially deacetylated derivative from chitin, still has many large acetyl side groups attached to the polymer chains which hinder the formation of a more organized struc-

ture (YUAN *et al.*, 2011). The diffraction peaks found in this study were close to those reported by Yen, Yang and Mau (2009) for chitosan obtained from the *Chionoecetes opilio* crab. In another report, Zhetcheva and Pavlova (2011) described that the chitosan obtained through the deacetylation step of chitin from a crab source (not informed the species) presented reflection peaks at (2θ) 10 and 20°. These values were also similar to those reported by Prashanth and Tharanathan (2007) who also attribute a semicrystalline structure to the chitosan obtained through deacetylation of chitin extracted from shrimp species.

The thermal decomposition profiles of the obtained samples were recorded using TGA technique (Figure 3). A first mass loss event can be observed for all analyzed

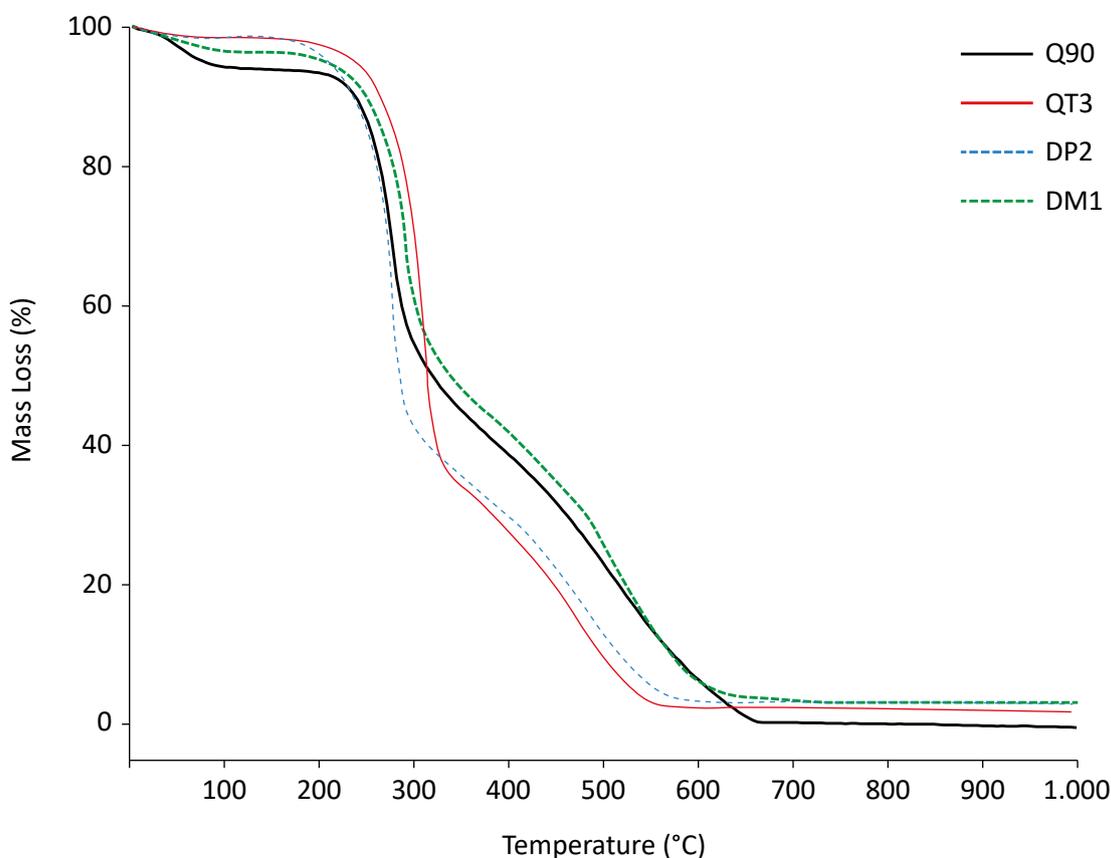


Figure 3 – Thermogravimetric analysis (TGA) curves of the materials obtained from the crab's residue: demineralized sample using 0.50 mol L⁻¹ solution of HCl for 30 min (DM1 - green); deproteinized sample using 1.0 mol L⁻¹ NaOH solution for 24 h (DP2 - blue); depigmented sample using 0.14 mol L⁻¹ NaClO solution for 8 h (QT3 - red) and deacetylated sample using 10 mol L⁻¹ NaOH solution for 90 minutes (Q90 - black).

samples ranging from room temperature up to 120°C and is related to the release of adsorbed water molecules. It can be inferred in the thermal profile of the demineralized material (DM1) that the mass loss process occurs less sharply in the non-oxidative event, which occurs in the temperature range of 190 to 340°C (PIRES *et al.*, 2013) in comparison to the oxidative event, which occurs between 340 and 650°C. The thermal decomposition of the DM1 material occurred less pronouncedly in the non-oxidative event, from 190 to 340°C, than in the oxidative event, which occurred in the temperature range of 350 to 650°C. This effect can be attributed to the presence of a greater amount of non-polymeric organic phase in the demineralized material (lipids and proteins) that decomposes differently from the polymeric material present in the structure of the crab's exoskeleton. In the non-oxidative process the thermal decomposition of glucopyranose units of the macromolecule occurs, including structural dehydration through the condensation of hydroxyl groups and fragmentation of C-C and C-O bonds, as well as the release of nitrogen atoms as nitrogen oxides (NO and NO₂) (PAULINO *et al.*, 2006). Subsequently, in the oxidative process, the remaining carbonaceous residue previously generated reacts with oxygen gas to release mainly CO₂ and CO (IQBAL *et al.*, 2011). After the thermal decomposition, a mass of 4.0% was identified. This residual mass is attributed mainly to calcium oxide (CaO) from the thermal decomposition of calcium carbonate (CaCO₃), initially present in the sample and not completely removed in the demineralization process. Considering this residual mass of 4.0% CaO, it was concluded that in the DM1 sample remained 7.1% of CaCO₃. In addition to the total 71.3% of CaCO₃ extracted in the demineralization process, a total of 78.4% of the initial constitution of *D. pagei* crab's residues formed by inorganic material was found. In the studies by Percot, Viton and Domard (2003) and Younes *et al.* (2014) the percentage of mineral residues found after the demineralization stage from shrimps, which have smaller inorganic content associated to the exoskeleton, was relatively lower than those found in this study, at 1.8 and 1.3%, respectively.

It is also observed that the deproteinized sample's TGA curve (DP2) presented a different thermal decomposition profile in relation to the chitin (QT3) and chitosan (Q90) curves in the temperature range of 325 to 670°C. This is due to the fact that the thermal decomposition

of the deproteinized material (DP2) is not only related to the mass loss of chitin, since the deproteinized material (DP2) still presents a quantity of non-polymeric organic content mainly in the form of pigments that are only removed under more oxidizing conditions (SEABRA; PEDROSA, 2010).

The thermal profile of QT3 sample exhibited three distinct mass loss events, in which the first event, occurring from room temperature up to 120°C, with mass variation recorded at 1.6% and is attributed to the release of adsorbed water molecules. The second mass loss event, occurring between 210 and 360°C, registered a mass reduction of 61.4% (non-oxidative mass loss step). Eventually, in the temperature range of 360 to 570°C, a mass reduction of 34.7% was observed (oxidative mass loss step). After the third mass loss event, a residual mass of 2.3% was observed.

Similarly, the thermal profile of Q90 sample also presents three mass loss steps, whereas the first one was registered from room temperature up to 120°C, with a mass reduction of 7.7%. The second event ranged from 220 to 330°C, with registered mass loss of 39.8%. The last event was recorded in the range of 330 to 670°C, with registered mass loss of 53.7%. After the third event, a residual mass of 0.3% was found. Assuming that the entire remained residue was associated to the thermally decomposed CaO phase originated from CaCO₃ that was not completely removed during demineralization step, it is estimated that a total mass of 0.52% of the produced chitosan is related to calcium carbonate.

The FTIR spectrum of the QT3 sample, shown in Figure 4, presents characteristic absorption bands of amide at 1,655 cm⁻¹ (C = O stretch), known as amide band I, at 1,561 cm⁻¹ (N-H deformation) called amide band II, and at 1,315 cm⁻¹ (angular deformation of the CO-NH bonds and the -CH₂ group occurring in the same region) called the amide band III, due to the deformation of the CO-NH group (CANELLA; GARCIA, 2001; LIU *et al.*, 2012). The band at 1,377 cm⁻¹ was attributed to the angular deformation of the -CH₃ groups. The band at 3,265 cm⁻¹ was attributed to the stretching of the N-H bond and the band observed at 3,454 cm⁻¹ was associated to the stretch of the O-H bond. Along with diffraction data, these results indicated that the QT3 sample is α-chitin.

Figure 5 shows the FTIR spectra of the chitosan samples obtained using different reaction times, in which the presence of the main bands were observed, as the one at $1,655\text{ cm}^{-1}$, corresponding to the C = O stretching and which refers to amide I, while the bands at $1,079\text{ cm}^{-1}$ and $1,034\text{ cm}^{-1}$ were associated with the stretching of the C-O bond (DUARTE *et al.*, 2002). The bands at $1,360$ and $1,317\text{ cm}^{-1}$ correspond to the axial deformation of the N-H bond and the band at $1,379\text{ cm}^{-1}$ refers to the angular deformation of the $-\text{CH}_3$ group (SOUZA; ZAMORA; ZAWADZKI, 2010).

The band at $1,590\text{ cm}^{-1}$ corresponds to the deformation of the $-\text{NH}$ group, while the bands observed between $1,600\text{--}1,670\text{ cm}^{-1}$ were associated with the stretching of the carbonyl bond. At $1,569\text{ cm}^{-1}$ region, the band corre-

sponds to the angular deformation of the $-\text{NH}_2$ group and at $1,153\text{ cm}^{-1}$ the signal was attributed to the angular deformation of the $-\text{COC}$ bond. At higher wavenumber region, bands at $2,926\text{ cm}^{-1}$ were associated with the stretching of the C-H bonds and the broad band at $3,400\text{ cm}^{-1}$ refers to the stretching of the O-H bond (BÖLGEN *et al.*, 2016). Through the FTIR technique, the hydrolysis of the α -chitin structure's acetylated groups can be verified by monitoring the reduction of the amide carbonyl stretching band at $1,655\text{ cm}^{-1}$ (KAYA; BARAN; KARAARSLAN, 2015). This observation is directly associated with the amount of chitin that was converted into chitosan through deacetylation process, since this process reduces the total amide bonds present and, hence, there was a reduction of the band intensity at $1,655\text{ cm}^{-1}$ as more chitin was converted into chitosan (YAGHOBI; HORMOZI, 2010). Thus, with

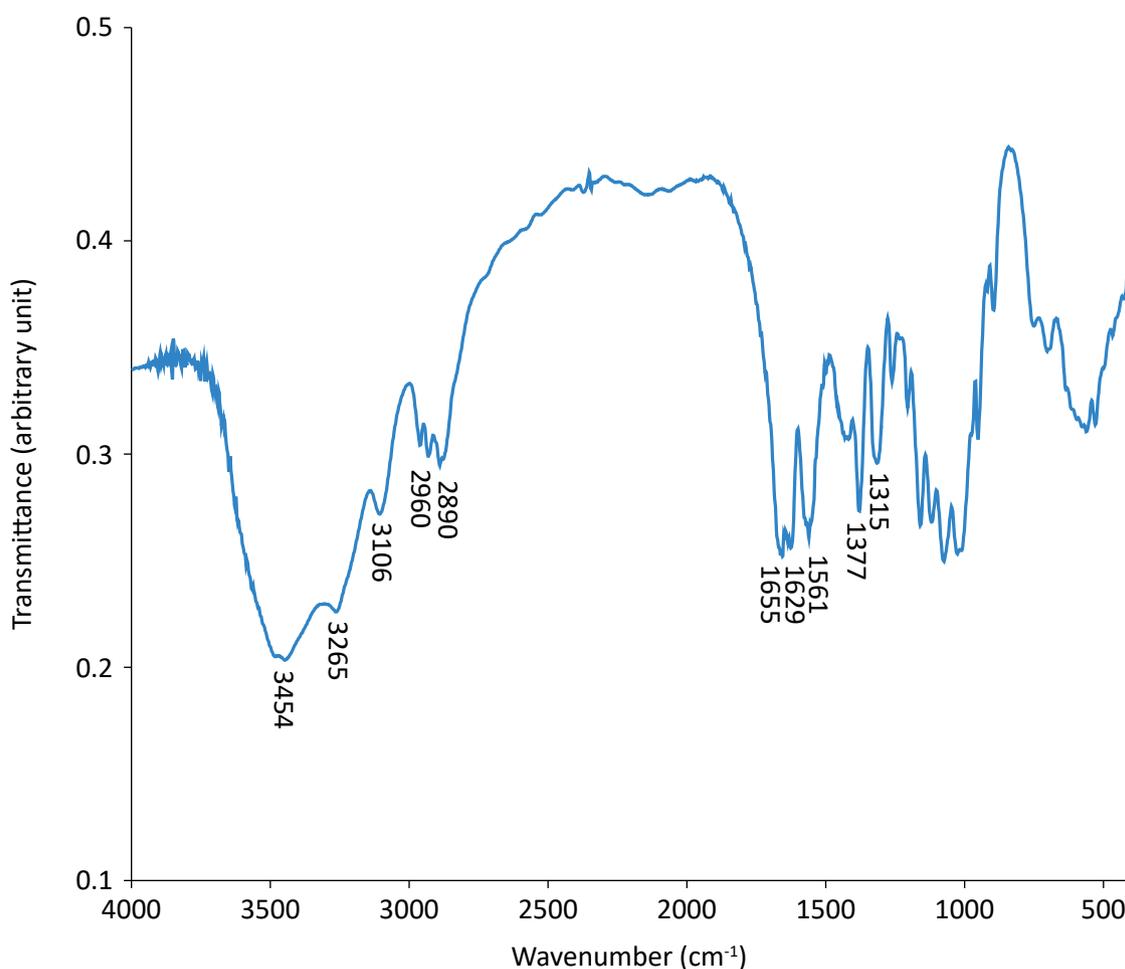


Figure 4 – The Fourier Transform Infrared (FTIR) spectrum of QT3 sample.

the increase in the samples' reaction time, the band at $1,590\text{ cm}^{-1}$ increases discretely its intensity, while in the same proportion the band $1,655\text{ cm}^{-1}$ decreases in intensity, indicating that the obtained chitosan samples possess different degrees of deacetylation.

From the absorbance values of the bands using the FTIR technique, it was possible to calculate the degree of deacetylation (DD) of the chitosan samples produced. The chitosan sample obtained through 60 minutes of reaction (Q60) had the DD of 68.4%, while the sample obtained after 90 minutes of reaction (Q90) exhibited a DD value of 81.9%. For the chitosan sample obtained after 120 minutes of reaction (Q120), a DD value of 77.6% was obtained. Hence, a growing tendency in DD with the increase of reaction time to pro-

duce chitosan can be observed. However, an increase in the DD when comparing the Q90 and Q120 samples was not observed. A plausible explanation lies on the fact that the vibrational band used to perform the calculations is very sensitive to the presence of moisture in the sample, which may have caused interference in the measured values of absorbance. Generally, there is a tendency to obtain around 70% of free -NH_2 groups during the deacetylation process in the first hour of reaction, when it occurs between 100 and 120°C , and in NaOH concentration of 10.0 and 12.5 mol L^{-1} and after one hour of reaction, deacetylation rate is decreased (YOUNES; RINAUDO, 2015). The results obtained in this process are close to those reported by Yen, Yang and Mau (2009) which applied similar experimental conditions in chitin's deacetylation process.

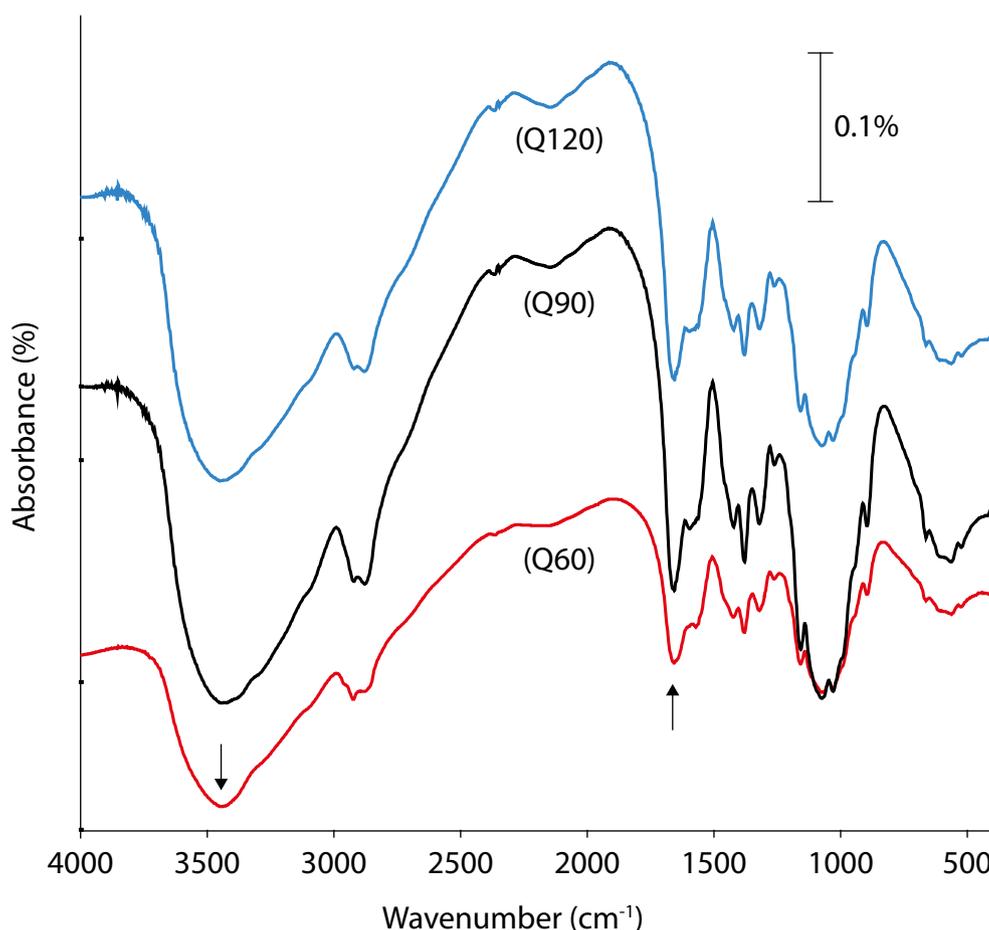


Figure 5 – The Fourier Transform Infrared (FTIR) spectra of chitosan samples obtained using reaction times of 60 (Q60 - red), 90 (Q90 - black) and 120 (Q120 - blue) minutes.

CONCLUSION

The experimental procedures used in this study, in order to obtain chitin and chitosan, showed that the residues of the freshwater crab *D. pagei* present high percentages of mineral content (78.4%). The analytical techniques employed in sample characterization showed the predominance of the α -chitin phase and that the final set of samples produced (Q60, Q90 and Q120) are chitosan samples. The thermal profile of all analyzed samples indicated a characteristic behavior of biopolymers, with the identification of a low content of impurities in the

chitosan samples obtained, while the infrared vibrational spectra indicated an increase of the deacetylation degree of the samples submitted to higher periods of reaction. The freshwater crab *D. pagei* has a large occurrence in other Brazilian regions; thus, it could be considered an important source of chitin and chitosan, especially in distant regions of the ocean, such as the Amazon region. The similar characteristic of chitin and chitosan compared to marine crustacean indicates that this species is a promising source of these biopolymers.

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REFERENCES

- AL-SAGHEER, F. A.; AL-SUGHAYER, M. A.; MUSLIM, S.; ELSABEE, M. Z. Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf. *Carbohydrate Polymers*, v. 77, n. 2, p. 410-419, 2009. <https://doi.org/10.1016/j.carbpol.2009.01.032>
- ARBIA, W.; ARBIA, L.; ADOUR, L.; AMRANE, A. Chitin extraction from crustacean shells using biological methods-a review. *Food Technology and Biotechnology*, v. 51, n. 1, p. 12-25, 2013.
- BARON, R. D.; PÉREZ, L. L.; SALCEDO, J. M.; CÓRDOBA, L. P.; SOBRAL, P. J. A. Production and characterization of films based on blends of chitosan from blue crab (*Callinectes sapidus*) waste and pectin from Orange (*Citrus sinensis Osbeck*) peel. *International Journal of Biological Macromolecules*, v. 98, n. 1, p. 676-683, 2017. <https://doi.org/10.1016/j.ijbiomac.2017.02.004>
- BENHABILES, M. S.; SALAH, R.; LOUNICI, H.; DROUICHE, N.; GOOSEN, M. F. A.; MAMERI, N. Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste. *Food Hydrocolloids*, v. 29, n. 1, p. 48-56, 2012. <https://doi.org/10.1016/j.foodhyd.2012.02.013>
- BILGIN, Ş.; FIDANBAŞ, Z. U. C. Nutritional properties of crab (*Potamon potamios* Olivier, 1804) in the lake of Egirdir (Turkey). *Pakistan Veterinary Journal*, v. 31, n. 3, p. 239-243, 2011.
- BOLAT, Y.; BILGIN, Ş.; GÜNLÜ, A.; IZCI, L.; KOCA, S. B.; ÇETINKAYA, S.; KOCA, H. U. Chitin-chitosan yield of freshwater crab (*Potamon potamios*, Olivier 1804) shell. *Pakistan Veterinary Journal*, v. 30, n. 4, p. 227-231, 2010.
- BÖLGEN, N.; DEMIR, D.; ÖFKELI, F.; CEYLAN, S. Extraction and characterization of chitin and chitosan from blue crab and synthesis of chitosan cryogel scaffolds. *Journal of the Turkish Chemical Society: A Chemistry*, v. 3, n. 3, p. 131-144, 2016. <https://doi.org/10.18596/jotcsa.00634>

- CAMPANA-FILHO, S. P.; BRITTO, D.; CURTI, E.; ABREU, F. R.; CARDOSO, M. B.; BATTISTI, M. V.; SIM, P. C.; GOY, R. C.; SIGNINI, R.; LAVALL, R. L. Extração, estruturas e propriedades de α -quitina e β -quitina. *Química Nova*, v. 30, n. 3, p. 644-650, 2007. <http://dx.doi.org/10.1590/S0100-40422007000300026>
- CANELLA, K. M. N. C.; GARCIA, R. B. Caracterização de quitosana por cromatografia de permeação em gel - influência do método de preparação e do solvente. *Química Nova*, v. 24, n. 1, p. 13-17, 2001. <http://dx.doi.org/10.1590/S0100-40422001000100004>
- CHAROENVUTTITHAM, P.; SHI, J.; MITTAL, G. S. Chitin extraction from black tiger shrimp (*Penaeus monodon*) waste using organic acids. *Separation Science and Technology*, v. 41, n. 6, p. 1135-1153, 2006. <https://doi.org/10.1080/01496390600633725>
- CHIAPPISI, L.; GRADZIELSKI, M. Co-assembly in chitosan-surfactant mixtures: thermodynamics, structures, interfacial properties and applications. *Advances in Colloid and Interface Science*, v. 220, n. 1, p. 92-107, 2015. <https://doi.org/10.1016/j.cis.2015.03.003>
- COSTA, E. S. *Rendimento e características físico-químicas da carne do camarão Macrobrachium amazonicum (Heller, 1862) e do caranguejo Dilocarcinus pagei (Stimpson, 1861)*. 83 f. Dissertação (Mestrado) – Instituto de Ciências Exatas e Tecnologia de Itacoatiara, Universidade Federal do Amazonas, Itacoatiara, 2015.
- DUARTE, M. L.; FERREIRA, M. C.; MARVÃO, M. R.; ROCHA, J. An optimised method to determine the degree of acetylation of chitin and chitosan by FTIR spectroscopy. *Biological Macromolecules*, v. 31, n. 1-3, p. 1-8, 2002. [https://doi.org/10.1016/S0141-8130\(02\)00039-9](https://doi.org/10.1016/S0141-8130(02)00039-9)
- HAMED, I.; ÖZOGUL, F.; REGENSTEIN, J. M. Industrial applications of crustacean by-products (chitin, chitosan, and chitooligosaccharides): A review. *Trends in Food Science & Technology*, v. 48, n. 1, p. 40-50, 2016. <https://doi.org/10.1016/j.tifs.2015.11.007>
- HENNIG, E. L. *Utilização de quitosana obtida de resíduos de camarão para avaliar a capacidade de adsorção de íons Fe³⁺*. 73 f. Dissertação (Mestrado) – Escola de Química e Alimentos, Universidade Federal do Rio Grande, Rio Grande, 2009.
- IQBAL, M. S.; AKBAR, J.; SAGHIR, S.; KARIM, A.; KOSCHELLA, A.; HEINZE, T.; SHER, M. Thermal studies of plant carbohydrate polymer hydrogels. *Carbohydrate Polymers*, v. 86, n. 4, p. 1775-1783, 2011. <https://doi.org/10.1016/j.carbpol.2011.07.020>
- KAYA, M.; BARAN, T.; KARAARSLAN, M. A new method for fast chitin extraction from shells of crab, crayfish and shrimp. *Natural Product Research*, v. 29, n. 15, p. 1477-1480, 2015. <https://doi.org/10.1080/14786419.2015.1026341>
- KAYA, M.; BARAN, T.; MENTES, A.; ASAROGLU, M.; SEZEN, G.; TOZAK, K. O. Extraction and characterization of α -chitin and chitosan from six different aquatic invertebrates. *Food Biophysics*, v. 9, n. 2, p. 145-157, 2014. <https://doi.org/10.1007/s11483-013-9327-y>
- KAYA, M.; DUDAKLI, F.; ASAN-OZUSAGLAM, M.; CAKMAK, Y. S.; BARAN, T.; MENTES, A.; ERDOGAN, S. Porous and nanofiber α -chitosan obtained from blue crab (*Callinectes sapidus*) tested for antimicrobial and antioxidant activities. *LWT - Food Science and Technology*, v. 65, n. 1, p. 1109-1117, 2016. <https://doi.org/10.1016/j.lwt.2015.10.001>
- KAYA, M.; MUJTABA, M.; BULUT, E.; AKYUZ, B.; ZELENKOVA, L.; SOFI, K. Fluctuation in physicochemical properties of chitins extracted from different body parts of honeybee. *Carbohydrate Polymers*, v. 132, n. 1, p. 9-16, 2015. <https://doi.org/10.1016/j.carbpol.2015.06.008>
- KUMARI, S.; ANNAMAREDDY, S. H. K.; ABANTI, S.; RATH, P. K. Physicochemical properties and characterization of chitosan synthesized from fish scales, crab and shrimp shells. *International Journal of Biological Macromolecules*, v. 104 (B), p. 1697-1705, 2017. <https://doi.org/10.1016/j.ijbiomac.2017.04.119>
- LARANJEIRA, M. C. M.; FÁVERE, V. T. Quitosana: biopolímero funcional com potencial industrial biomédico. *Química Nova*, v. 32, n. 3, p. 672-678, 2009. <http://dx.doi.org/10.1590/S0100-40422009000300011>

LIU, J.; PU, H.; LIU, S.; KAN, J.; JIN, C. Synthesis, characterization, bioactivity and potential application of phenolic acid grafted chitosan: A review. *Carbohydrate Polymers*, v. 174, n. 1, p. 999-1017, 2017. <https://doi.org/10.1016/j.carbpol.2017.07.014>

LIU, S.; SUN, J.; YU, L.; ZHANG, C.; BI, J.; ZHU, F.; QU, M.; JIANG, C.; YANG, Q. Extraction and characterization of chitin from the beetle *Holotrichia parallela* Motschulsky. *Molecules*, v. 17, n. 4, p. 4604-4611, 2012. <https://doi.org/10.3390/molecules17044604>

MAGALHÃES, C.; CAMPOS, M. R.; COLLINS, P. A.; MANTELATTO, F. L. Diversity, Distribution and Conservation of Freshwater Crabs and Shrimps in South America. In: KAWAI, T.; CUMLERLIDGE, N. (Orgs.). *A global overview of the freshwater decapod crustaceans*. Cham: Springer International Publishing, 2016. p. 303-322.

MATO GROSSO DO SUL. Secretaria do Meio Ambiente, do Planejamento e Ciência e Tecnologia do Mato Grosso do Sul (SEMACE). *Resolução nº 3, de 28 de fevereiro de 2011*. Disciplina aspectos referentes à captura, transporte, estocagem, comercialização e cultivo de iscas vivas no Estado de Mato Grosso do Sul. Mato Grosso do Sul: SEMACE, 2011a. Available at: <<http://www.imasul.ms.gov.br/wp-content/uploads/sites/74/2015/11/Resolu%C3%A7%C3%A3o-SEMACE-N%C2%BA-3-DE-28-02-2011-iscas-vivas.-Alterado-RES.-SEMACE-n.-022-de-25-de-agosto-de-2011.pdf>>. Accessed on: November 29, 2018.

_____. Secretaria do Meio Ambiente, do Planejamento e Ciência e Tecnologia do Mato Grosso do Sul (SEMACE). *Resolução nº 22, de 25 de Agosto de 2011*. Altera disposições da Resolução SEMACE nº 3, de 28 de fevereiro de 2011 referentes à captura, transporte, estocagem, comercialização e cultivo de iscas vivas no Estado de Mato Grosso do Sul. Mato Grosso do Sul: SEMACE, 2011b. Available at: <<https://www.legisweb.com.br/legislacao/?id=139771>>. Accessed on: November 29, 2018.

MATOS, S. R. M. *Caracterização dos resíduos do camarão Litopenaeus vannamei e avaliação de suas potencialidades como flavorizante*. 68 f. Dissertação (Mestrado) – Departamento de Engenharia de Pesca, Universidade Federal do Ceará, Fortaleza, 2005.

MENDES, A. A.; OLIVEIRA, P. C.; CASTRO, H. F.; GIORDANO, R. L. C. Aplicação de quitosana como suporte para a imobilização de enzimas de interesse industrial. *Química Nova*, v. 34, n. 5, p. 831-840, 2011. <http://dx.doi.org/10.1590/S0100-40422011000500019>

OGAWA, M.; MAIA, E. L.; FERNANDES, A. C.; NUNES, M. L.; OLIVEIRA, M. E. B.; FREITAS, S. T. Resíduos do beneficiamento do camarão cultivado: obtenção de pigmentos carotenoides. *Ciência e Tecnologia de Alimentos*, v. 27, n. 2, p. 333-337, 2007. <http://dx.doi.org/10.1590/S0101-20612007000200022>

OLIVEIRA, B. S.; NUNES, M. L. Avaliação de quitosana de caranguejo-uçá (*Ucides cordatus*) como biofilme protetor em caju. *Scientia Plena*, v. 7, n. 4, p. 1-6 (041501), 2011.

PAULINO, A. T.; SIMIONATO, J. I.; GARCIA, J. C.; NOZAKI, J. Characterization of chitosan and chitin produced from silkworm chrysalides. *Carbohydrate Polymers*, v. 64, n. 1, p. 98-103, 2006. <https://doi.org/10.1016/j.carbpol.2005.10.032>

PERCOT, A.; VITON, C.; DOMARD, A. Optimization of chitin extraction from shrimp shells. *Biomacromolecules*, v. 4, n. 1, p. 12-18, 2003. <https://doi.org/10.1021/bm025602k>

PIRES, N. R.; CUNHA, P. L. R.; MACIEL, J. S.; ANGELIM, A. L.; MELO, V. M. M.; PAULA, R. C. M.; FEITOSA, J. P. A. Sulfated chitosan as tear substitute with no antimicrobial activity. *Carbohydrate Polymers*, v. 91, n. 1, p. 92-99, 2013. <https://doi.org/10.1016/j.carbpol.2012.08.011>

PRASHANTH, K. V. H.; THARANATHAN, R. N. Chitin/chitosan: modifications and their unlimited application potential - an overview. *Trends in Food Science & Technology*, v. 18, n. 3, p. 117-131, 2007. <https://doi.org/10.1016/j.tifs.2006.10.022>

RINAUDO, M. Chitin and chitosan: properties and applications. *Progress in Polymer Science*, v. 31, n. 7, p. 603-632, 2006. <https://doi.org/10.1016/j.progpolymsci.2006.06.001>

- SAHARIAH, P.; MÁSSON, M. Antimicrobial Chitosan and Chitosan Derivatives: A Review of the Structure-Activity Relationship. *Biomacromolecules*, v. 18, n. 11, p. 3846-3868, 2017. <https://doi.org/10.1021/acs.biomac.7b01058>
- SAKTHIVEL, D.; VIJAYAKUMAR, N.; ANANDAN, V. Extraction of chitin and chitosan from mangrove crab *Sesarma plicatum* from Thengaithittu estuary pondicherry southeast coast of India. *International Journal of Pharmacy e Pharmaceutical Research*, v. 4, n. 1, p. 12-24, 2015.
- SANTOS, M. C.; CIRILO, A. T. O.; NUNES, M. L. Determinação do grau de desacetilação de quitosana obtida de camarão "Saburica" (*Macrobrachium jelskii*, Miers, 1877). *Scientia Plena*, v. 7, n. 9, p. 1-4 (091501), 2011.
- SEABRA, L. M. J.; PEDROSA, L. F. C. Astaxanthin: structural and functional aspects. *Revista de Nutrição*, v. 23, n. 6, p. 1041-1050, 2010. <http://dx.doi.org/10.1590/S1415-52732010000600010>
- SILVA, B. M. S.; MORALES, G. P.; GUTJAHR, A. L. N.; FAIAL, K. C. F.; CARNEIRO, B. S. Bioaccumulation of trace elements in the crab *Ucides cordatus* (Linnaeus, 1763) from the macrotidal mangrove coast region of the Brazilian Amazon. *Environmental Monitoring and Assessment*, v. 190, n. 4, p. 214, 2018. <https://doi.org/10.1007/s10661-018-6570-1>
- SOUZA, K. V.; ZAMORA, P. G. P.; ZAWADZKI, S. F. Esferas de quitosana/Fe na degradação do corante Azul QR-19 por processos foto-Fenton utilizando luz artificial ou solar. *Polímeros*, v. 20, n. 3, p. 210-214, 2010. <http://dx.doi.org/10.1590/S0104-14282010005000035>
- VÁZQUEZ, J. A.; RODRÍGUEZ-AMADO, I.; MONTEMAYOR, M. I.; FRAGUAS, J.; GONZÁLEZ, M. P.; MURADO, M. A. Chondroitin sulfate, hyaluronic acid and chitin/chitosan production using marine waste sources: Characteristics, applications and eco-friendly processes: A review. *Marine Drugs*, v. 11, n. 3, p. 747-774, 2013. <https://doi.org/10.3390/md11030747>
- WU, T.; ZIVANOVIC, S.; DRAUGHON, F. A.; CONWAY, W. S.; SAMS, C. E. Physicochemical Properties and Bioactivity of Fungal Chitin and Chitosan. *Journal of Agricultural Food Chemistry*, v. 53, n. 10, p. 3888-3894, 2005. <https://doi.org/10.1021/jf048202s>
- YAGHOBI, N.; HORMOZI, F. Multistage deacetylation of chitin: Kinetics study. *Carbohydrate Polymers*, v. 81, n. 4, p. 892-896, 2010. <https://doi.org/10.1016/j.carbpol.2010.03.063>
- YEN, M. T.; YANG, J-H.; MAU, J-L. Physicochemical characterization of chitin and chitosan from crab shells. *Carbohydrate Polymers*, v. 75, n. 1, p. 15-21, 2009. <https://doi.org/10.1016/j.carbpol.2008.06.006>
- YEO, D. C. J.; NG, P. K. L.; CUMBERLIDGE, N.; MAGALHÃES, C.; DANIELS, S. R.; CAMPOS, M. R. Global diversity of crabs (Crustacea: Decapoda: Brachyura) in freshwater. *Hydrobiologia*, v. 595, n. 1, p. 275-286, 2008. <https://doi.org/10.1007/s10750-007-9023-3>
- YOUNES, I.; HAJJI, S.; FRACHET, V.; RINAUDO, M.; JELLOULI, K.; NASRI, M. Chitin extraction from shrimp shell using enzymatic treatment. Antitumor, antioxidant and antimicrobial activities of chitosan. *International Journal of Biological Macromolecules*, v. 69, n. 1, p. 489-498, 2014. <https://doi.org/10.1016/j.ijbiomac.2014.06.013>
- YOUNES, I.; RINAUDO, M. Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Marine Drugs*, v. 13, n. 3, p. 1133-1174, 2015. <https://doi.org/10.3390/md13031133>
- YUAN, Y.; CHESNUTT, B. M.; HAGGARD, W. O.; BUMGARDNER, J. D. Deacetylation of Chitosan: Material Characterization and *in vitro* Evaluation via Albumin Adsorption and Pre-Osteoblastic Cell Cultures. *Materials*, v. 4, n. 8, p. 1399-1416, 2011. <https://doi.org/10.3390/ma4081399>
- ZHETCHEVA, V. D. K.; PAVLOVA L. P. Synthesis and characterization of a decavanadate/chitosan complex. *Turkish Journal of Chemistry*, v. 35, n. 2, p. 221-223, 2011. <https://doi.org/10.3906/kim-1006-718>

