

# Valorization of shrimp waste by chemical extraction of chitin and chitosan

Chadia IHAMOUCHEM<sup>1\*</sup>, Hocine DJIDJELLI<sup>1</sup>, Amar BOUKERROU<sup>1</sup>

<sup>1</sup>Department of Processes Engineering, Laboratory of Advanced Polymer Materials, University of Bejaia, 06000 Bejaia, Algeria

Corresponding author\*: Chadia.benmerad@univ-bejaia.dz

Received: 02 January 2022; Accepted: 26 January 2022; Published: 27 January 2022

## Abstract

*The aim of the present work is the recovery of co-products from shrimp shells by isolation of chitin and chitosan. Chitin is the major structural component of the exoskeleton of invertebrates. Chitosan, the deacetylated form of chitin, exhibits many biological activities including antifungal, antibacterial properties, wound-healing properties, and tumor inhibition. Its biological and physico-chemical properties make it an attractive biopolymer for highly targeted applications. The shrimp shells are widely used as raw material to isolate chitin, which is done by, the chemical or enzyme method. In this study, the choice is made on the first method which requires the dissolution of minerals, by acid treatment and extraction of the proteins by a basic treatment, followed by a stage of bleaching to remove pigments. Chitosan, it is obtained by deacetylation of chitin. The isolated substances were characterized by several techniques: chemical characterization, infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and the X-ray diffraction. FTIR analysis confirmed the conversion of chitin to chitosan with deacetylation of 78.7%. The presence of a high crystalline portion in the chitin was observed by XRD. Regarding the glass transition, the estimated humidity 6% of the chemical characterization in chitin played the role of a plasticizer; therefore, there was a decrease in Tg.*

**Keywords:** Chitin, Shrimp Shells, Chitosan, N-deacetylation

## I. Introduction

The oceans cover more than two-thirds of the earth's surface and a great diversity of organisms live and proliferate there. The management of marine litter is problematic. In the case of shrimp, more than 75% of its weight is rejected, the equivalent of more than 16,000 tons per year [1]. The management of this waste generates costs that affect the performance of processing companies.

There are many current solutions for the management of shrimp waste. Some prefer burying or calcining, an expensive and environmentally harmful operation. Their transformation into compost, of course, is less polluting, but not profitable. Its use in animal feed, but expensive, unprofitable, and therefore little used. As a last resort, the discharge of this waste directly into the sea causes unwanted organic pollution [2].

The research studies on the recovery of shrimp waste led to the discovery of chitin. A major structural component of the exoskeleton of marine invertebrates, mainly crustaceans, is another route that appears to be more profitable, as this compound can be transformed into chitosan which has good

commercial value [3]. Exhibits many biological activities including antifungal, antibacterial properties, wound-healing properties, and tumor inhibition. Their biological, physical, and chemical properties make it an attractive biopolymer for highly targeted applications

The objective of our work falls within the framework of the recovery and management of shrimp waste. The choice of this product lies in its richness in chitin. The physicochemical properties and the wide variety of biological activities make chitin and its derivatives the biopolymers of choice for many applications such as biotechnology, medicine, food, environment, etc... [4].

The recovery of chitin takes place in two stages: demineralization and deproteinization of the shells thus forming a protein matrix and containing insoluble mineral salts, mainly calcium carbonates. As for chitosan, it is obtained by deacetylation of chitin in 40% to 50% sodium hydroxide solution under pressure, at temperatures above 100 °C [5].

## I. Material and methods

### I.1. Shrimp shell powder

The shrimp shells were recovered at the port of Boumerdes, Algeria these shells have undergone several pre-treatment namely: Washing, Drying and Grinding



Figure 1: Shrimp shell before and after grinding

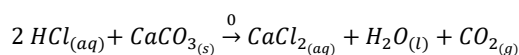
## II. Experimental techniques

### II.1. Chitin extraction process

Although chitin was identified before cellulose, it initially received less attention in scientific research than the latter. Chitin performs a function similar to that of cellulose in plants by acting as supportive and protective material. With its crystal structure and association with proteins, it prevents the internal organs of crustaceans from swelling in seawater [6]. Many methods have been developed to extract chitin from the shells of aquatic invertebrates. Chemical extraction consists of removing mineral elements (demineralization), proteins (deproteinization), and color (bleaching) [7].

#### a) Demineralization

Demineralization is carried out with 1N hydrochloric acid, for 30 min, at room temperature and with a solid/solvent ratio of 1/10 w/v, to transform the insoluble minerals of the shells into soluble salts. Calcium carbonate, the main mineral compound in the shell, reacts with HCl to form calcium chloride, water and carbon dioxide as described in the following reaction:



Most of the other minerals present react similarly and give soluble salts in the presence of acid. The salts formed can be separated from the chitin by simple filtration followed by washing. During the demineralization process, unwanted foams can form due to the production of carbon dioxide.

#### b) Deproteinization

The deproteinization is carried out in a reflux flask, in the presence of a 3% NaOH solution and with a solid/solvent percentage of 1/10 w/v. The duration of the basic treatment is of the order of 120 minutes at a temperature of 110 °C, to dissolve a larger part of the proteins.

#### c) whitening

For industrial applications, the chitin obtained from crustacean resources must be decolorized after the acid and alkali treatments. The bleaching was done in a solution of ethanol for 5 min with a solid/ratio of 1/15 w/v. Several washes are necessary between each step

### II.2. Chitosan obtaining

While the extraction of chitin is easily achievable today, the most delicate step remains deacetylation, which requires sufficient substitution of the acetyl groups to result in chitosan. The chitin obtained is treated with a solution of sodium hydroxide (NaOH) 45% by mass at a temperature of 110 °C, for 1 hour and 30 minutes in a ratio of 1: 15w/v to remove some or all of the acetyl groups. The product obtained is then filtered, washed several times with distilled water and dried in an oven at 40 °C for 24 hours.

### II.3. Characterization techniques

#### II.3.1. Chemical characterizations

##### a) Dry matter

1 to 2 g of sample is taken and weighed in a cup of known weight. The dish is placed for 24 h in an oven at 105 °C, and then weighed after cooling for 30 min. The experiment is performed in triplicate [8, 9]. The dry matter content in chemical extraction products is given by the following formula:

$$DE(\%) = \frac{M_2 - M_0}{M_1 - M_0} .100$$

DE (%) is the part of dry extract,  $M_0$  the weight of the cup,  $M_1$  the wet weight, and  $M_2$  the final dry weight.

##### b) Moisture content

The water content of the samples is determined according to the AFNOR standard (NF ISO 712). In a glass dish, previously dried for 1 hour in an oven at 100-105 °C and cooled in desiccators, place 5 g of the product to be analyzed. Leave to dry in the oven at 100-105 °C until constant mass. Weigh the quantity of dry residue after cooling in desiccators [8, 9].

$$\text{Moisture}(\%) = \frac{m(\text{humid sample}) - m(\text{dry sample})}{m(\text{humid sample})} .100$$

##### c) Ash content

To determine the mineral content of the samples, approximately 1 g of the sample is weighed into a sheet of aluminum foil of known weight. It is folded up and placed in an oven for at least 5 hours at 600 °C. After cooling, it is weighed again. The weight of

residual ash is equated with the mineral content. Each measurement is repeated three times [8, 9].

$$\text{Ash} (\%) = \frac{M_2 - M_0}{M_1 - M_0} \cdot 100$$

Ash% is the ash content,  $M_0$  is the weight of the container,  $M_1$  and  $M_2$  are the weights before and after incineration respectively.

#### d) Purity and soluble residues

The purity of the product must be equal to or greater than 95%. Place 5 g of the sample to be analyzed in 100 ml of bi-distilled water and stir for 2 minutes. Filter after cooling through a tight filter. Evaporate the filtrate and dry at 100-105 ° C. The soluble matter content (SM %) must not exceed 5%. It is calculated using the following formula [3]. The degree of purity corresponds to the complementary part.

$$\text{SM} (\%) = \frac{m(\text{sample after drying})}{m(\text{sample before test})} \cdot 100$$

$$\text{Purity} (\%) = 100 - \text{SM}$$

#### II.3.2. FTIR spectroscopy Analysis

FTIR spectroscopy can highlight the appearance or disappearance of certain bands very significantly during extraction. The transmission spectra were carried out using an infrared spectrometer model SHIMADZU FTIR-8400S and were obtained in a wavenumber domain extending from 4000 to 400  $\text{cm}^{-1}$  on KBr pellets, with a spectral resolution of 4  $\text{cm}^{-1}$ .

#### II.3.3. Differential Scanning Calorimetry Analysis (DSC)

The apparatus used is of the TA Instruments, TGA Q10 type. The experiments were carried out on samples of mass ranging from 5 to 10 mg, placed in an aluminum crucible and heated in an inert nitrogen medium with a heating rate of the order of 10 °C/min and in a temperature range from 20 °C to 250 °C.

#### II.3.4. X-ray diffraction (XRD)

X-ray diffraction analyzes were performed on an X Pert Pro Panalytical type apparatus using the  $K\alpha_1$  line of copper with wavelength  $\lambda = 1.540598 \text{ \AA}$ . The diffractograms were recorded from 0° to 70° (2 $\theta$ ) with a step of 0.01°.

The crystallinity index, denoted X (%), was calculated from the XRD spectra, according to the method of Focher *et al.* [10], according to the formula below.

$$X (\%) = \frac{I_{110} - I_{am}}{I_{110}} \cdot 100$$

With :  $I_{110}$ , (à  $2\theta = 20^\circ$  dans le plan de réflexion (110)),  $I_{am}$  (autour de  $2\theta = 12^\circ$ ), le pic représentatif de la région amorphe

### III. Results and discussion

#### III.1. Chemical characterizations

Chemical compositions such as dry matter content, moisture content, mineral or ash content, purities and soluble residues were determined for the chitin, and chitosan samples. Regarding the ash content, the test was carried out on the two samples chitin and chitosan in addition to the raw material (i.e without any prior treatment). The various results found are shown in Table 1.

**Table 1:** The chemical composition of the extraction products

Chemical compositions (%)	Chitin	Chitosan	Raw material
Dry matter	90,83	90,13	/
Humidity	6,5	5	/
Ash	0,24	0,24	28,54
purity	95,37	97,57	/
Soluble residues	2,43	4,63	/

From the results of Table 1, we note that:

- The ash content of the raw material exhibits a very high rate which is 28% while the chitin and chitosan samples register only 0.24%. This drastic decrease is due to the demineralization process which consists of the removal of minerals. This means that the demineralization step was successful.
- The moisture content of chitin (6.5%) is slightly higher than that of chitosan (5%). Moisture absorption in chitin is due not only to the presence of amide I (-NH) and II (-NH<sub>2</sub>) groups that are also found in chitosan (amide II), but also to the presence of groups acetyl. These two groups (amide and acetyl) have free electron pairs with the ability to bind water vapor in the air.
- The rate of soluble residues in chitosan is slightly higher than that of chitin, which is quite expected. Chitosan has the exceptional characteristic of being positively charged which allows it to react with all negatively charged compounds (H<sub>2</sub>O) and to fix them by ionic bonds [11]. This particularity gives it many properties and applications. Unlike chitin which is insoluble in most solvents.
- The rate of purity recorded for chitosan is 97.57% while that of chitin is 95.37%. This difference is due to the additional treatment that the chitosan has undergone, which is deacetylation by prolonged alkaline treatment, which resulted in better purification due to both treatment and repeated washing.

### III.2. FTIR spectroscopy analysis

The FT-IR spectra of the chitin and chitosan samples are shown in Figure 2. From this Figure, we notice there are some absorption bands that are identical in the two spectra and other bands appear in the spectrum of chitin only whereas they disappear in that of chitosan [12].

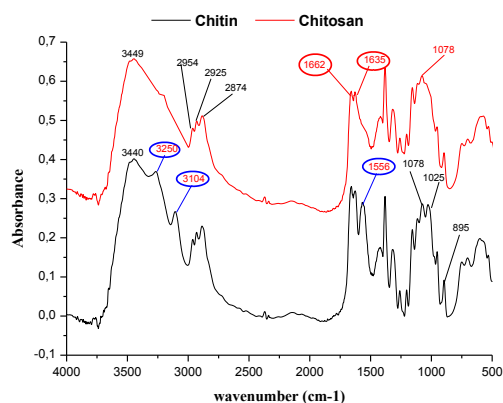


Figure 2: FTIR spectrum of chitin and chitosan

- The two spectra show a wide band between 3100-3500 cm<sup>-1</sup> to more particularly at 3440 cm<sup>-1</sup> which correspond to the –OH stretching vibrations
- The chitin spectrum also shows two peaks at 3250 and 3104 cm<sup>-1</sup> which correspond to intermolecular hydrogen bonds and amide II –NH stretching vibrations. While these peaks do not exist in the spectrum of chitosan, which reflects a decrease in hydrogen bonds, therefore a more amorphous state is justified by the results of the XRD and that the deacetylation has taken place and has been successful.
- Peaks between 1960 -1860 cm<sup>-1</sup> correspond to the stretching vibrations of –CH and –CH<sub>2</sub>.
- The peaks appearing at 1662, 1635cm-1 and the peak at 1556 cm<sup>-1</sup> in the chitin spectrum are due respectively to the C=O stretching vibrations of the groups in amide I and the amide II [13]. Thus, the appearance of two peaks at 1662 and 1635 cm<sup>-1</sup> shows that the chitin used is an α-chitin. However, α-chitin is the most stable and abundant orthorhombic system with aligned antiparallel macromolecules forming a regular crystal structure) [14]. The first peak corresponds to the vibrations between the C-N and C=O groups of the amide I linked by a hydrogen bridge to the OH groups. The second corresponds to an amorphous state, where the detected vibrations correspond to the bonds between the amide I and the C=O group [15].
- The absence of the peak at 1556 cm<sup>-1</sup> in the spectrum of chitosan shows that the latter is very deacetylated [11].
- There are also two peaks at 1078 and 1025 cm<sup>-1</sup> which correspond to the asymmetric C-O-C elongation vibrations of the glucosidic cycle and

another peak at 895 cm-1 which corresponds to the β (1-4) glucosidic bonds in the polysaccharide.

- The absence of the peak at 1540 cm<sup>-1</sup> which corresponds to the low or absence of protein impurities in the two spectra, demonstrates the purity of the chitin used and that the deproteinization was successful.

### III.3. Degree of acetylation (DA) and degree of deacetylation (DD)

- The degree of acetylation (DA) is the one of the most important parameters to observe at the level of chitin is its degree of N-acetylation. The principle is based on the ratio of the areas between the characteristic bands of N-acetylglucosamine amine, chitin, and reference bands. According to the bibliography, several formulas are proposed [6]. In our case, we opted for the formula of M. R Kasai et al. [16].

$$DA \% = \frac{\left(\frac{A_{1655}}{A_{3450}}\right)}{1,33} \cdot 100$$

With: A<sub>1655</sub>: Absorbance of C=O groups in the amide. I A<sub>3450</sub>: Absorbance of OH hydroxyl groups. The factor 1.33 represents the ratio (A<sub>1655</sub>/A<sub>3450</sub>) for a chitosan entirely N-acetylated.

- The degree of deacetylation (DD) is one of the main parameters characterizing chitosan, it corresponds to the degree of free –NH<sub>2</sub> groups in the chitosan molecule and is given by the following formula [16].

$$DD \% = NH_2 \% = [1 - DA\%] \cdot 100$$

After carrying out the necessary calculations, we have got the following results.

Table 2: Degree of acetylation and deacetylation

sample	DA (%)	DD (%)
Chitin	82.59	37.90
Chitosan	28.25	78.7

From the values in Table 5, it can be seen that the deacetylation of the chitin gave an acceptable degree of deacetylation (DD ≈ 79%). This leads us to conclude that the chemical treatments carried out were very successful and allowed us to extract chitin and chitosan from the shell powder of the shrimp, similar to the commercialized products found on the market.

### III.4. X-ray diffraction (XRD)

Figure 3 shows the XRD spectra of chitin and chitosan. X-ray diffraction spectra of chitin and chitosan show the presence of several peaks. Each peak is associated with an atomic plane; these planes

are designated by miller indices (h, k, and l). For example, the reflection at  $2\theta = 9.41^\circ$ , is relative to the plane (020), the reflection at  $2\theta = 19.5^\circ$  corresponds to the plane (110) and the reflection at  $2\theta = 26.40^\circ$  is relative to the plane (130).

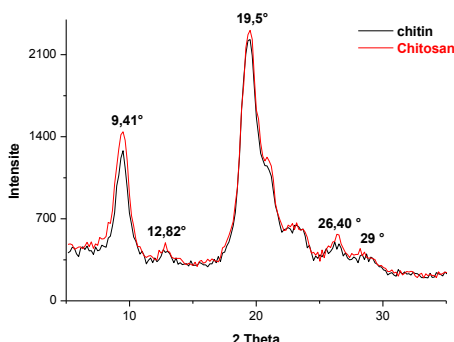


Figure 3: DRX spectrum of chitin and chitosan

It can be seen that the first two peaks (at 9.41 and 19.5°) are very intense and they correspond to the proportion of crystalline zones within the structure of the sample, and three other peaks at 12.82, 26.40 and 29° have very low intensities. Our results are similar to those obtained by Zhou et al. [17] with pure commercial chitin. Other researchers Entsar et al. [18] have shown that  $\alpha$ -chitin has a strong reflection at  $2\theta = 9-10^\circ$  and  $2\theta = 20-21^\circ$  and a minor reflection at  $2\theta = 26.4^\circ$ . We can therefore conclude that the characteristic peaks of pure chitin appear in our sample prepared by chemical means. We also note that the intensity of the peaks corresponding to chitosan is slightly higher than that of chitin. The rate of crystallinity, denoted X (%), was calculated from the XRD spectra, according to the method of Focher et al. [10]. The results obtained are shown in Table 3.

Table 3: The rate of crystallinity of chitin, chitosan

Samples	X (%)
chitin	62,47
chitosan	54,46

According to the results of Table 3, it can be seen that the crystallinity index of chitosan which is 54.46% is lower than that of chitin which is estimated at 62.47%. This reduction, according to J. Machida [19], is attributed to prolonged acid and basic treatments which tend to reduce crystallinity as well as to deacetylation which involves the reduction of N-acetyl groups, demonstrated by Revol and Marchessault [20].

**III.5. Differential enthalpy analysis (DSC)**

Figures 4 show the DSC thermograms of the chitin and chitosan samples. We note that the two thermograms exhibit an endothermic peak between 100°C and 200°C which is due to the fusion of these

two biopolymers, and more precisely the melting temperature of chitin was estimated at 198°C and that of chitosan 186°C. This difference can be attributed on the one hand to the difference in the crystallinity rates [21], because a rate of 62.5% for chitin was recorded against 54.5% for chitosan calculated from the XRD. This result is confirmed by the heat of fusion  $\Delta H_f$  calculated from the DSC thermograms. On the other hand, the presence of impurity was estimated at 5% in chitin according to the chemical characterization carried out. This caused an increase in the melting temperature

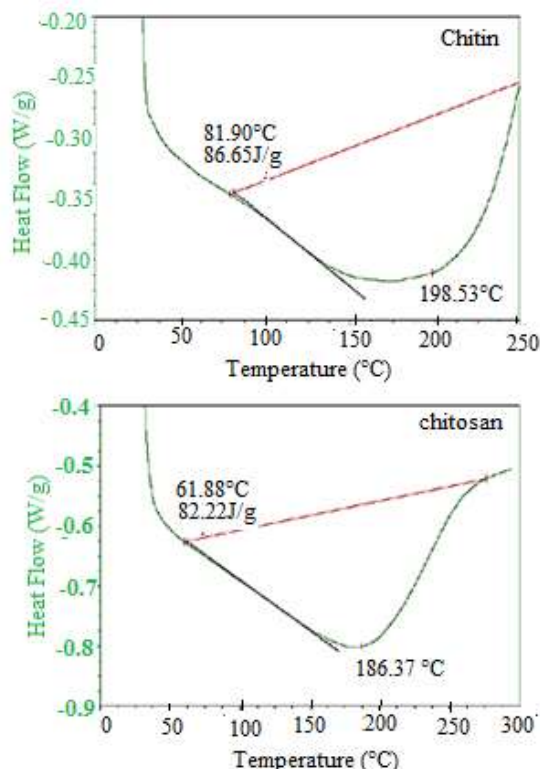


Figure 4: Representation of the melting temperature of chitin and chitosan

The glass transition temperatures (Tg) of chitin and chitosan are shown in Figures 5 and 6.

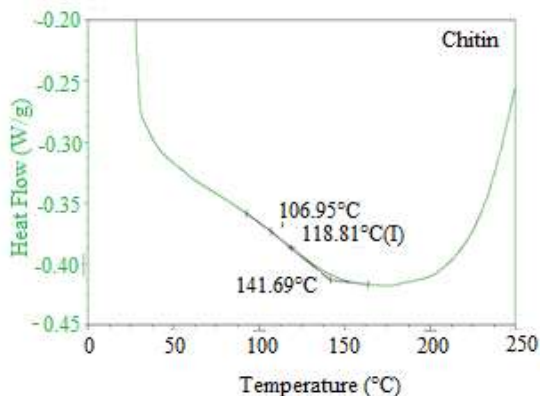
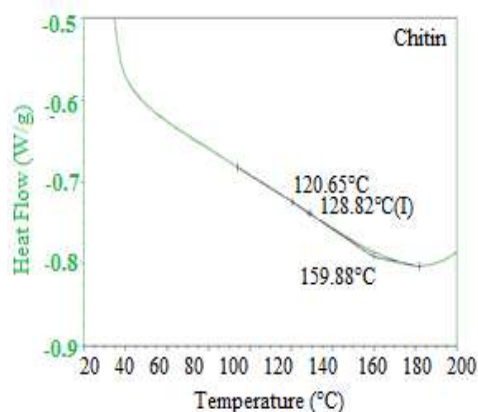


Figure 5: The glass transition temperatures of chitin



**Figure 6:** The glass transition temperatures of chitosan

In the chitin sample, its Tg is observed at 141°C. The decrease in the Tg of chitin compared to chitosan (160°C) would be due to the suppression of certain hydrogen bonds between the macromolecular chains, caused by the presence of a small quantity of water molecule (Rate of humidity estimated at 6.5% according to the results of the chemical characterization) which plays an important role as a plasticizer. Thus, increasing the flexibility of the chains, which makes their sliding a little easier.

#### IV. Conclusions

The demonstration of the extraction was examined by different analytical techniques, namely chemical characterization, IRTF analysis, X-ray diffraction and thermal analysis by DSC. The various results revealed that: The raw material exhibits a very high ash content of 28%, while the chitin sample registers only 0.24%. This means that the demineralization step was successful. IRTF analysis confirmed the conversion of chitin into chitosan, justified by the substitution of acetyl groups for chitin by amine groups for chitosan. The degree of acetylation and deacetylation for chitin (DA=82.59%) and chitosan (DD=78.7%) respectively, lead us to conclude that the chemical treatments carried out were successful and allowed us to extract chitin and chitosan similar to the commercial products found on the market. The presence of a large crystalline part in the chitin observed by XRD confirms the increase in the melting temperature of chitin (198 °C) compared to chitosan (186 °C) observed by DSC. Concerning the glass transition, the presence of water molecules estimated at 6% according to the results of the chemical characterization in chitin played the role of a plasticizer, thus increasing the flexibility of the chains that leads to sliding a little and consequently a reduction in his Tg.

#### Conflict of interest

The authors declare that they have no conflict for financial interests or personal relationships that how can influence the work reported in this paper.

#### References

- [1] X. Xiong Chang, N. M. Mubarak, S. A. Mazari, A. S. Jatoi, A Ahmad, M. Khalid, R. Walvekar, E.C. Abdullah, R Rao Karri, M.T.H Siddiqui, S. Nizamuddin. A review on the properties and applications of chitosan, cellulose, and deep eutectic solvent in green chemistry. *Journal of Industrial and Engineering Chemistry*, 104, 362-380, 2021. <https://doi.org/10.1016/j.jiec.2021.08.033>
- [2] M. Rinaudo, Chitin and chitosan: Properties and applications. *Progress in Polymer Science*. 31, 603-632, 2006 <https://doi.org/10.1016/j.progpolymsci.2006.06.001>
- [3] M.N.V.R. Kumar. A review chitin and chitosan applications. *Reactive and Functional Polymers*. 46 (1), 1-27, 2000. [https://doi.org/10.1016/S1381-5148\(00\)00038-9](https://doi.org/10.1016/S1381-5148(00)00038-9)
- [4] M. Abhinaya, R. Parthiban, P. Senthil Kumar, N. Vo Dai-Viet A review on cleaner strategies for extraction of chitosan and its application in toxic pollutant removal. *Environmental Research* 196, 110996, 2021. <https://doi.org/10.1016/j.envres.2021.110996>
- [5] A. J. da Silva Lucas, E. Quadro Oreste, H. L. Gouveia Costa, H. M. Lopez , C D Medeiros Saad , C. Prentice. Extraction, physicochemical characterization, and morphological properties of chitin and chitosan from cuticles of edible insects. *Food Chemistry* 343, 128550, 2021. <https://doi.org/10.1016/j.foodchem.2020.128550>
- [6] B. Taser , H. Ozkan, A. Adiguzel, T. Orak, M. Ozkan Baltaci, M. Taskin Preparation of chitosan from waste shrimp shells fermented with *Paenibacillus jamilae* BAT1. *International Journal of Biological Macromolecules* 183, 1191–1199, 2021. <https://doi.org/10.1016/j.ijbiomac.2021.05.062>
- [7] D. Raabe, P. Romano, C. Sachs, H. Fabritius, A. Al-Sawalmih, S. B. Yi, G. Servos et H. G. Hartwig. «Microstructure and crystallographic texture of the chitinprotein network in the biological composite material of the exoskeleton of the lobster *Homarus americanus*». *Materials Science and*

- Engineering: A 421, 143-153, 2006. <https://doi.org/10.1016/j.msea.2005.09.115>
- [8] R. Priyadarshi, J.W. Rhim. Chitosan-based biodegradable functional films for food packaging applications. *Innovative Food Science and Emerging Technologies* 62, 102346, 2020. <https://doi.org/10.1016/j.ifset.2020.102346>
- [9] M. Pakizeh, A. Moradi, T. Ghassemi. Chemical extraction and modification of chitin and chitosan from shrimp shells. *European Polymer Journal* 159, 110709, 2021. <https://doi.org/10.1016/j.eurpolymj.2021.110709>
- [10] B. Focher, P.L. Beltrame, A. Naggi, G. Torri. Alkaline N-deacetylation of chitin enhanced by flash treatments. Reaction kinetics and structure modifications. *Carbohydr Polym*, 12(4), 405-418, 1990. [https://doi.org/10.1016/01448617\(90\)90090-F](https://doi.org/10.1016/01448617(90)90090-F)
- [11] C. Ben amar. Chitosan: Properties, Modifications and Food Nanobiotechnology. *Procedia Manufacturing* 46, 652–658, 2020. <http://creativecommons.org/licenses/by-nc-nd/4.0/>.
- [12] H. El Knidri, R. Belaabed, A. Addaou, A. Laajeb, A. Lahsini. Extraction, chemical modification and characterization of chitin and chitosan. *International Journal of Biological Macromolecules* 120, 1181–1189, 2018. <https://doi.org/10.1016/j.ijbiomac.2018.08.139>
- [13] Q. Luo, Y. Wang, Q. Hana, L. Jib, H. Zhang, Z. Feib, Y. Wang. Comparison of the physicochemical, rheological, and morphologic properties of chitosan from four insects. *Carbohydrate Polymers* 209 (2019) 266–275. <https://doi.org/10.1016/j.carbpol.2019.01.030>
- [14] A. A. Vaidya, I. Hussain, M. Gaugler, D. A. Smith. Synthesis of graft copolymers of chitosan-poly(caprolactone) by lipase catalysed reactive extrusion. *Carbohydrate Polymers* 217, 98-109, 2019. <https://doi.org/10.1016/j.carbpol.2019.03.081>
- [15] R. L. Lavall, O. B.G. Assis, P. Sérgio, F. Campana.  $\beta$  – Chitin from the pens of *Loligo* sp : Extraction and characterization. *Bioresource Technology* Vol.98, (13), 2465-2472, 2007. <https://doi.org/10.1016/j.biortech.2006.09.002>
- [16] M. R. Kasaai. Various methods for determination of the degree of N-acetylation of chitin and chitosan: a review. *J Agric Food Chem*, 57(5), 1667-76, 2009. <https://doi.org/10.1021/jf803001m>
- [17] D Zhou, L. Zhang, S. Guo. Mechanisms of lead biosorption on cellulose / chitin beads. *Water Research* 39(16), 3755-3762, 2005. <https://doi.org/10.1016/j.watres.2005.06.033>
- [18] S. Abdou Entsar, S.A Nagy Khaled, Z.Maher Elsabee. Extraction and characterization of chitin and chitosan from local sources. *Bioresource Technology* 99(5), 1359-1367, 2008. <https://doi.org/10.1016/j.biortech.2007.01.051>
- [19] J. Machida, S. Suenaga, M. Osada. Effect of the degree of acetylation on the physicochemical properties of  $\alpha$ -chitin nanofibers. *International Journal of Biological Macromolecules* 155, 350–357, 2020. <https://doi.org/10.1016/j.ijbiomac.2020.03.213>
- [20] J.F Revol, R.H Marchessaul. In vitro chiral nematic ordering of chitin crystallites. *Int J Biol Macromol* 15(6), 329-335, 1993. [https://doi.org/10.1016/0141-8130\(93\)90049-R](https://doi.org/10.1016/0141-8130(93)90049-R)
- [21] H. Zhou, H. Tong, J. Lu, Y. Cheng, F. Qian, Y. Tao, H. Wang. Preparation of bio-based cellulose acetate/chitosan composite film with oxygen and water resistant properties. *Carbohydrate Polymers* 270 118381, 2021. <https://doi.org/10.1016/j.carbpol.2021.118381>