ISOLATION AND CHARACTERIZATION OF CHITIN FROM SHELLS OF THE FRESHWATER CRAB POTAMON ALGERIENSE

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Abstract

This work presents, for the first time, the extraction and characterization of chitin from the shell of the freshwater crab species Potamon algeriense with a standardized and revised chemical method. Chitin and chitosan were isolated following demineralization, deproteinization, decolouration (raw chitin), and deacetylation (chitosan). After boiling, drying, and grinding, 62.12% of the ground shell was obtained. A yield of 40.92% was obtained after demineralization of ground crab shell, while after the deproteinization process 8.74% was obtained. After decolourization, 8.27% of raw chitin was obtained, and the final amount of chitosan extracted from the crab shells was approximately 5.89%. We also characterized the isolated chitin by determining its physicochemical properties using X-ray powder diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and thermogravimetric analysis (TGA).

Keywords: *Chitin, chitosan, deacetylation, freshwater crab, Potamon algeriense*

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1. Introduction

Chitin is the second most abundant biopolymer in nature, after cellulose. It is usually isolated from the exoskeletons of invertebrates, insects, marine diatoms, sponges, mollusks, coralline algae, cell walls of certain fungi, and crustaceans like crabs, shrimps, and lobsters by chemical processes using strong acids and bases [1–6]. The colour of chitin is light yellow to brown, and the appearance of chitin is as flocculence or a filiform solid. In addition, chitin is not soluble in water [7]. Chitin does not have a single chemical structure, but many. It includes several polysaccharides composed of N-acetyl-β-D-glucosamine units (from 50 to 100%) and D-glucosamine units (from 0 to 50%).

Structurally, chitin is a straight-chain polymer composed of β-1,4-N-acetylglucosamine, and it is classified into three different natural polymorphs, α-, β-, and γ-chitin [8,9], with α-chitin being the most common in nature and having a structure of antiparallel chains, usually isolated from the exoskeleton of crustaceans and more particularly from shrimps, crabs, and lobsters [5]. β-chitin can be obtained from squid pens. It has intra-sheet hydrogen bonding by parallel chains [10,11]. Meanwhile, γ-chitin, found in yeast and the cell walls of certain fungi, has not been completely identified. It has been proposed that it is a mixture of two parallel chains and one antiparallel chain. [11,12] have suggested that γ-chitin can be a combination of α and β structures rather than a different polymorph.

Because chitin has a compact structure, it is insoluble in most solvents [13,14]. Therefore, chemical modifications of chitin are performed [15]. The most common derivative is chitosan, a straight-chain polymer of glucosamine and N-acetylglucosamine, hydrophilic, natural, cationic, nontoxic biopolymer derived from partial N-deacetylation of chitin [16–18].

The last three decades have seen active research into potential usual applications of chitin and its derivatives, mainly chitosan. Because of its biodegradability, biocompatibility, and non-toxicity, chitosan has a wide range of applications in different fields, e.g., cosmetics, agriculture, food, pharmacy, biomedicine, the paper industry, paint and textile industries, wastewater treatment, wound healing, and drug delivery systems [17–43]. Chitosan and chitosan oligomers are also known for their biological activities, such as their antimicrobial [41–55], antitumor [39,40], and hypocholesterolemic functions [41].

The carapace waste of crustaceans is constituted mainly of 30–50% calcium carbonate, 30–40% protein, and 20–30% chitin. Nevertheless, these constituents are changeable, depending on the species and seasons [49]. To quote some examples, the shell waste of the snow crab *Chionoecetes opilio* and the northern prawn *Pandalus borealis* contains approximately 17–32.2% chitin [50–59]. It has been estimated that the chitin content of the blue crab was 14%. It was also determined that the grey shrimp *Crangon crangon* contains 17.8% chitin [1], while the speckled shrimp *Metapenaeus Monoceros* contains 4.5–7% chitin [52].

The freshwater crab, *Potamon algeriense* [53], belongs to the family of the Potamidae, which is the largest of all freshwater crab families and comprises 95 genera and over 505 species [54,55]. *P. algeriense* can be found in North Africa, exclusively in three countries, Morocco, Algeria, and Tunisia [54]. In Morocco, the species has been reported from the north in the watershed of the Oued Laou near Chefchaouen, from the Northeast in the watershed of Moulouya, and from the Middle Atlas in the Oued Oum Rbia watershed near Khenifra. Despite their wide distribution, the population of *P. algeriense* has not been commercially evaluated. In fact, freshwater crabs are also an important source of chitin, like the other crustaceans.

The aim of this study was to extract and determine the yield of chitin and chitosan from the carapace of *P. algeriense*, known as the freshwater crab of Maghreb, and it has not

been economically evaluated, besides its wide range of applications in numerous industrial areas.

2. Materials and Methods

2.1. Chitin and Chitosan Extraction

Specimens of the freshwater crab *Potamon algeriense* [53] from Oued Zegzel northeast of Morocco were used in this study (Fig. 1 and Fig. 2). For extracting chitin and chitosan, crabs were boiled for 15 min in order to take them out from their carapaces. The shells were scraped free of soft tissue, cleaned, rinsed, and dried at 60°C for 24 hours. The shells were ground with a Retsch mill (model Brinkmann Rmo) to obtain a coarse powder and were then sieved to a 350-μm diameter. The extraction of chitin and chitosan from the crab carapace was performed with three repetitive analyses. Chitosan was extracted from the shells by means of mineralization, deproteinization, decolouration, and deacetylation.

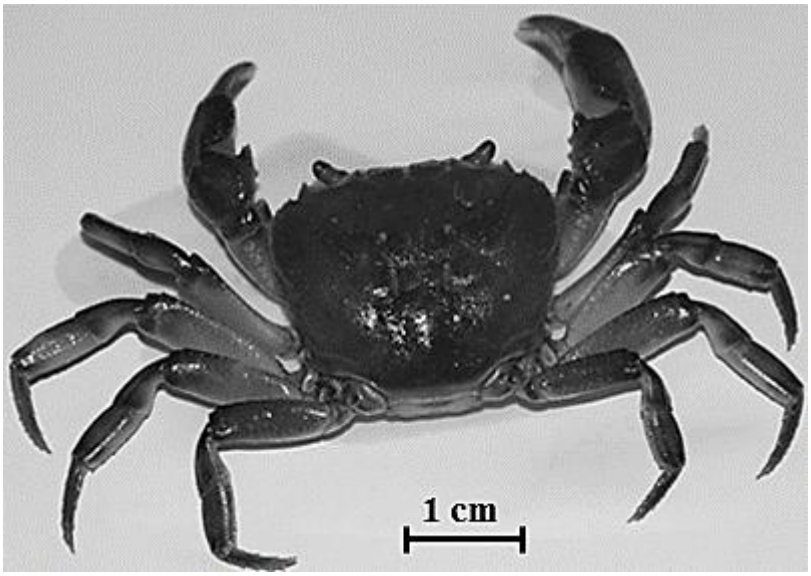

Figure 1. The freshwater crab *Potamon algeriense.*

The ground shells were then soaked very slowly (to avoid overflow of the sample due to the massive emission of CO_2 gas) in a 1 N HCl solution for six hours at room temperature to remove calcium salts (demineralization), with a solid/solvent ratio of 1:15 (w/v) [56]. The decalcified shell was collected on Whatman paper filter in a Buchner funnel. The resulting solid was washed with deionized water until it was neutralized. Then, the demineralized samples were dried and weighed.

Chitin deproteinization was carried out under standard autoclaving conditions (15 psi/121°C). The demineralized shell was treated with aqueous sodium hydroxide solution (3%) for 20 min at 15 psi/121°C, and the solid/solvent ratio was 1:10 (w/v) [49]. The absence of proteins was indicated by the absence of colour of the medium at the last treatment. The resulting solution was then washed to neutrality, filtered, dried, and weighed as mentioned above (Fig. 3).

For the purpose of decolouration, the obtained chitin residue was treated further with 0.32% sodium hypochlorite solution for 10 min, with a solid/solvent ratio of 1:10 (w/v) [56]. Following decolouration, the discoloured chitin was collected and washed with

deionized water until it was neutralized. It was then dried at 60°C for 24 h to obtain purified discoloured crab chitin.

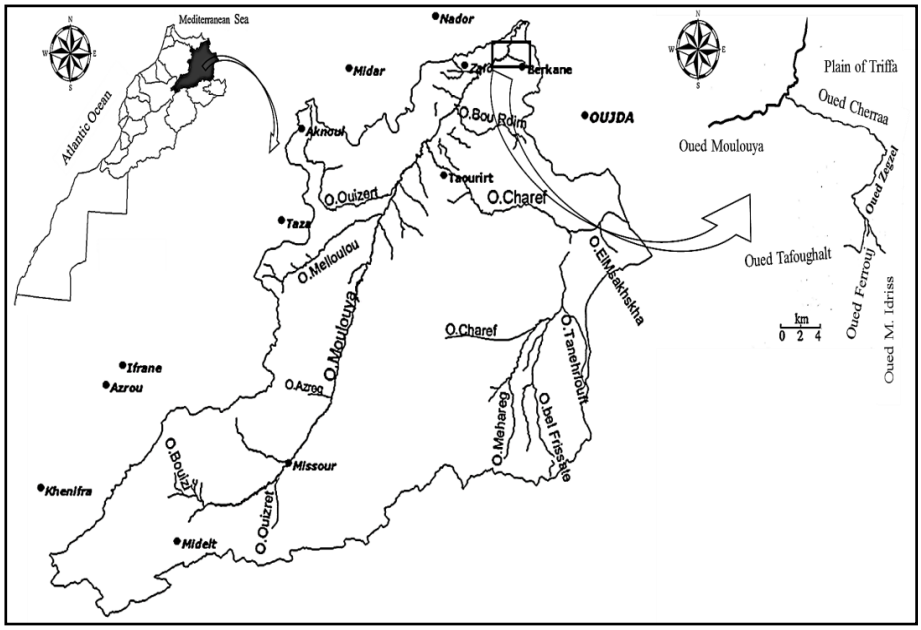

Figure 2. Area of the study in Oued Zegzel, Morocco.

For deacetylation, purified crab chitin was treated under the conditions of 15 psi/121°C with 50% NaOH for 30 min, and a solid/solvent ratio of 1:15 (w/v) was used [57]. The resulting chitosan was filtered, washed, dried, and weighed. The yield of chitosan extracted from crabs was then calculated. All data collected from this work were subjected to statistical analysis using Statistical Package for the Social Sciences (SPSS) [58].

2.2. Characterization of Chitin

2.2.1. Fourier Transform Infrared Spectroscopy (FTIR)

The infrared spectra were registered in a Fourier transform infrared (FTIR) spectrometer (Nicolet Magna, Nicolet Analytical Instruments, Madison, WI) connected to a PC with Omnic software (Thermo Electron Corp) for data processing. The analyses were directly performed on finely powdered *Potamon algeriense* material. The samples were prepared in KBr pellets at a concentration of 5% (w/w) [59]. They were placed into the crystal cell and the cell was clamped into the mount of the FTIR spectrometer. In this work, we used a range of 500–4500 cm^{-1} then the automatic signal gain was collected and rationed against a background spectrum recorded from the clean empty cell.

2.2.2. X-Ray Diffraction (XRD)

The degree of crystallinity and the size of the crystallites were determined by means of the X-ray diffraction (XRD) method. The same finely ground powder samples as used for FTIR were used for this analysis. The polymorphism of the crystals in the samples was determined by an X-ray diffractometer (Phillips) with 30 kV and 40 mA Cu Kα radiations. Sample analyses were carried out in the 20–60° range of the 2Θ angle, with step sizes of 0.020° and a point measurement time of 2 s.

2.2.3. Thermogravimetric (TG) Analysis

Thermogravimetric (TG) analysis of this material was carried out with a Perkin Elmer TGA 7 apparatus with a platinum sample holder using Pyris software for data handling. Measurements were performed in a nitrogen atmosphere at a heating rate of 15°C min^{-1}. The samples were heated up to at least 700°C, starting from 50°C.

3. Results and Discussion

3.1. Chitin and Chitosan Extraction

Three repetitive analyses were performed for the purpose of calculating the quantity of chitosan extracted from the shells of *P. algeriense*. Dried and ground crab shells in the wet weight state were quantified with a ratio of 62.12%. The results are shown in Fig. 3 and Table 1.

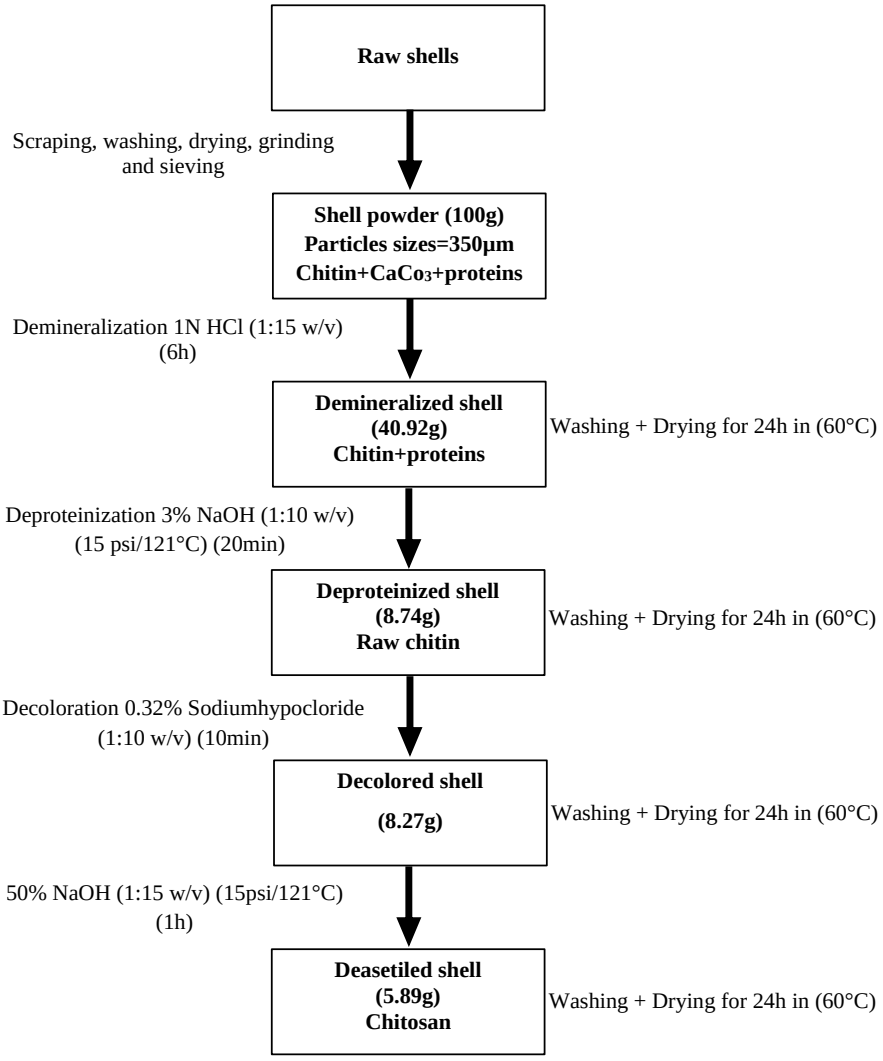

Figure 3. Isolation process of chitin and preparation of chitosan from crab shells.

About 22 crab species from 4500 species over the globe have been commercially evaluated [54,60]. In ecological investigations, crabs are considered biological indicators [61]. Numerous studies have concentrated on the biological, chemical, and ecological aspects of freshwater crabs [61–65], but rare studies have been carried out on the extraction of chitin and chitosan [66].

The quantity of chitosan is estimated to be approximately 1.560 million tons throughout the world [67]. The shells of crustaceans currently present the main industrial source for extraction of this biopolymer. One hundred billion tons of chitin are generated from arthropods, mollusks, and the cell walls of certain fungi every year. Cauchie et al. (1997) estimated that the annual production of this polysaccharide in freshwater ecosystems is only approximately 600 million tons [67]. Knoor (1984) stated that chitin is the most under-exploited polysaccharide around the globe. There is currently a total of 238 genera and 1.476 species of known freshwater crabs from 14 families around the world [53] that could be exploited as sources of chitosan.

The chemical process of isolating chitin and chitosan from crab shells after drying and grinding results in a yield of purified chitin of 8.27% and a yield of chitosan of 5.89%. The results are presented in Fig. 3 and Table 1.

Table 1. The yield (%) of chitosan isolated from crab shells.

Isolation process	% Yield
Wet weight	100.00
Weight of boiled shell	64.85
Weight of dried shell	62.32
Weight of ground shell	62.12
Dry weight	
Ground shells	100.00
Demineralized shells	40.92
Deproteinized shells	8.74
Decoloured shells (Raw chitin)	8.27
Deasetiled shells (Chitosan)	5.89

In the present manuscript, the yield after boiling the specimens was quantified as 64.85%, which was statistically significant (P>0.05) according to the yield of dried and ground freshwater crab shell. These results were in accordance with those acquired with the sand crab *Portunus pelagicus* [67, 68]. Approximately 8.27% of chitin was found in the ground shells of *P. algeriense*. The results obtained by Mol [69] showed a yield of around 3–6% chitin. Chakrabarti (2002) reported that the brown shrimp *Metapenaeus monoceros* contains about 4.5–7% chitin in shell waste [62], while Hertrampf and Piedal-Pascual (2000) declared that the content of chitin extracted from the snow crab *Chionoecetes opilio* was nearly 10.6% [70]. The quantity of chitin was 26.6% in *Chionoecetes opilio* [71] and 17.8% in the grey shrimp *Crangon crangon* [72]. Tharanathan (2003) reported that 14% of chitin was found in blue crab, while in *Chinoecetes opilio* and *Pandalus borealis* chitin content ranged from 17 to 32.2% [50,51]. Cho (1998) declared that the difference in the potential amount of chitin and chitosan between various crabs depends on the species and the seasons [49].

Chitosan is a biopolymer resulting from alkaline deasetilation of chitin. It is found mainly in the exoskeleton of arthropods and is quite easy to isolate methodologically. In this paper, 5.89% of chitosan was extracted from the freshwater crab *P. algeriense*. Due

to the easier and inexpensive method of collecting and capturing freshwater crabs compared to that of marine species, freshwater crabs could be raised as a source of chitin and they may also present a new alternative fishing material in freshwater ecosystems. For all of these reasons, freshwater crustaceans, especially crabs, could be commercially evaluated by producing chitin and chitosan because of the ease of processing for chitin extraction.

3.2. Characterization of Chitin and Chitosan

3.2.1. Fourier Transform Infrared Spectroscopy

The infrared (IR) spectra of chitin from the crab species *potamon algeriense* are shown in Fig. 4. These spectra presented peaks at 890 cm–1, which is due to the C–H bonds of the anomeric carbon. Several authors used this band to characterize the configuration of the anomeric centre from the glucopyranosicyclic residues of chitin:C-H axial at 891±7 cm–1 and C–H equatorials at 844±8 cm–1 atoms [72]. Therefore, the chitin of *potamon algeriense* is characterized by the β-configuration in the anomeric centre (C_1) of this polysaccharide.

On the other hand, these spectra were characterized by two wide peaks. The first wide peak lies between 913 and 1108 cm^{-1}. This range corresponds to C=O, so this result is also indicative of chitin [73]. The second wide peak is between 1242 and 1570 cm^{-1}. This interval includes the significant amide bands that correspond to the amide II of N–H and the amide III of C–N.

Finally, we found two low peaks (2801–2889 cm^{-1}) corresponding to amide B (2800–2990 cm^{-1}) [75,76]. The results indicate a system containing amino-polysaccharide chitin alongside proteins.

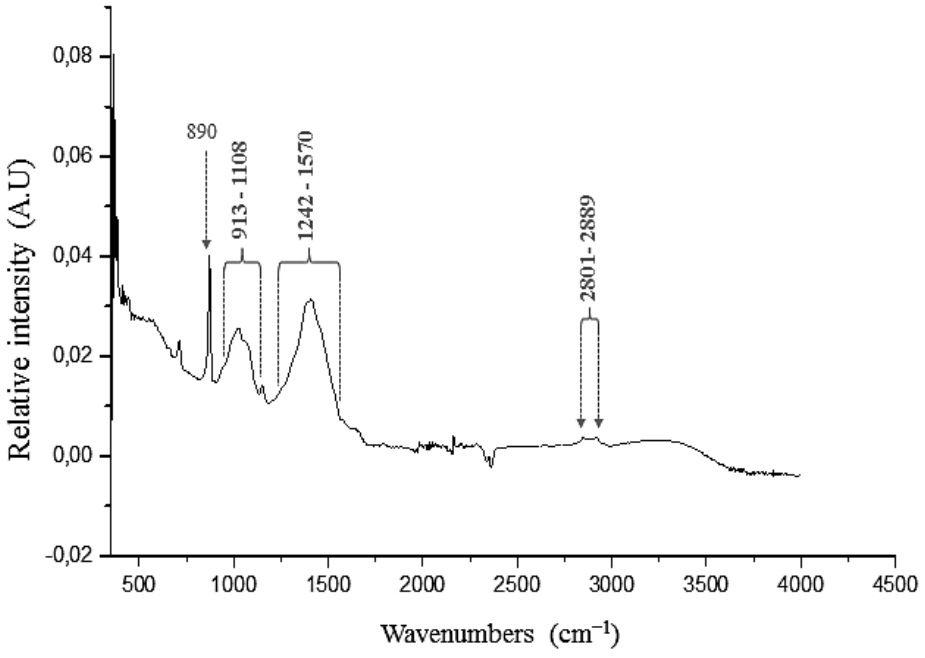

Figure 4. Fourier transform infrared (FTIR) spectra of chitin obtained from *potamon algeriense* in the range of 4500–400 cm^{-1}.

3.2.2. X-Ray Diffraction

To detect the orientation of the crystal structures and to understand the functional properties of the organic matrix components in *potamon algeriense*, the finely ground shell powder samples were analysed by XRD. The XRD pattern of *potamon algeriense* shell is shown in **Fig. 5**. The XRD analysis revealed that the biggest diffraction face intensity is at 2θ=29.4. This intensity corresponds to the rhombohedra calcite. Rahman et al. (2014) confirmed that this diffraction angle (2θ=29.4) in the calcite (104) indicates the presence of Mg–calcite [74].

The comparison of our XRD diffraction results with different types of calcite (012, 140, 110, 113, 202, 018, 116, 122, 211) shows that is similar to the strongest faces of calcite. Therefore, we showed different crystal surfaces in our case. We found that the crystalline components in the shell of *potamon algeriense* exhibited the characteristics of chitin and collagen calcite planes.

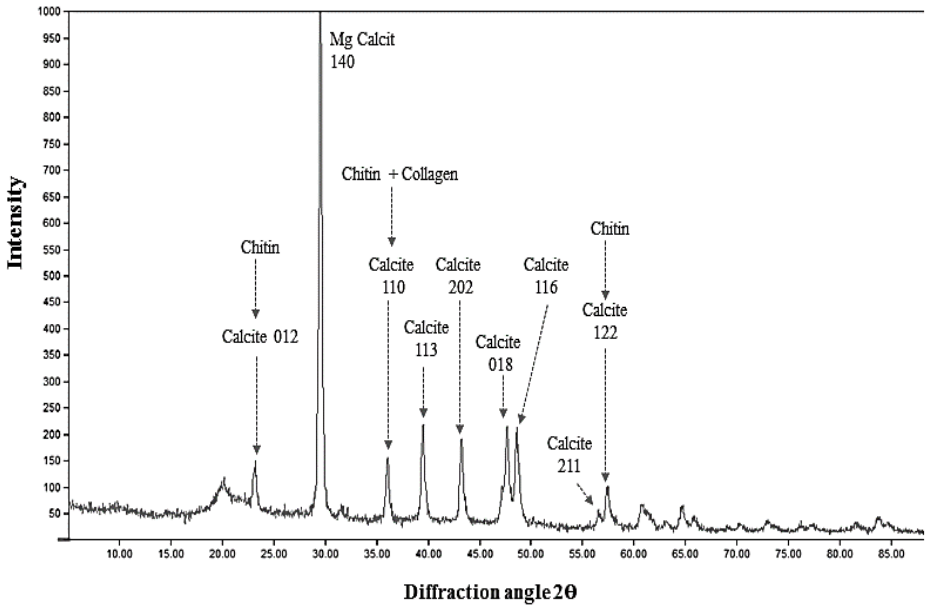

Figure 5. X-ray diffraction (XRD) analysis of chitin obtained from *potamon algeriense*. The diffraction scan identifies the mineral form of calcium carbonate with calcitic crystal planes, which were nucleated by chitin and collagen matrices.

3.2.3. Thermogravimetric Analysis

Concerning the chitin obtained from *potamon algeriense*, the mass loss was observed in four stages (**Fig. 6**). In the first stage, there was a mass loss of 5%. This loss was due to water evaporation within the structure. In the second stage, mass loss amounted to 15.3%. The mass loss observed at this stage was due to the beginning of the decomposition of the chitin molecules. In the third stage, mass loss was 22.8%, corresponding to the continued decomposition of chitin. During the fourth step, we observed the biggest mass loss rate (24.8%). The mass loss observed at this stage was due to the decomposition of calcite in the collagen calcite. These results show the good thermal stability of the extracted chitin.

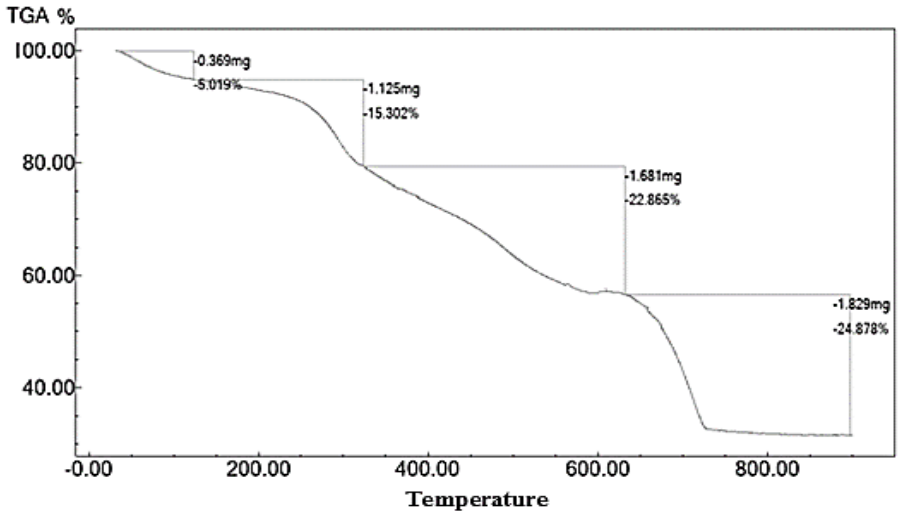

Figure 6. Thermogravimetric analysis (TGA) of chitin obtained from *potamon algeriense*.

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