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Comparative Physicochemical Study of Shellfish-Derived Chitosan from Southern Benin and Commercial Chitosan for PEM Fuel Cell Applications

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Abstract

Biopolymers, particularly chitosan derived from chitin, are attracting growing interest due to their versatility and environmental benefits. One notable application is the development of proton exchange membranes (PEM) for fuel cells, which offer a cleaner and more sustainable energy solution. This study aims to promote the use of local resources rich in biopolymers. For this purpose, a comparative analysis was performed on the physicochemical properties, melting point, X-ray diffraction, and degree of deacetylation of chitosan extracted from shrimp shells, compared to commercial chitosan. The main results of this study show, on the one hand, that the degree of deacetylation (DDA) of chitosan extracted from shrimp shells in the laboratory is 54.08%, while that of commercial chitosan is 97%. The study also revealed that the extraction yield of chitosan from shrimp shells was approximately 33.33% of the initial mass.

Keywords

Chitosan, Degree of Deacetylation (DDA), Extraction Yield, X-Ray Diffraction (XRD), Biopolymer

1. Introduction

Polysaccharides are naturally occurring biopolymers that serve essential structural

and functional roles in both plants and animals [1]. Among these, chitin, the second most abundant natural polysaccharide after cellulose, and its primary derivative, chitosan, have attracted considerable scientific attention in recent decades [2] [3]. This growing interest is reflected in the exponential rise in research publications focused on chitosan and its diverse applications [4]. Chitin and chitosan are typically extracted from the exoskeletons of marine crustaceans such as crabs and shrimp, as well as from certain terrestrial or freshwater sources like snail shells [5]. However, in many regions, crustacean shell waste from the fishing and seafood industries is discarded into the ocean, where it poses environmental concerns due to its slow biodegradation rate [6]. At the fishing port of Cotonou, shrimp shells are usually discarded at waste collection point and left unused. According to a local saleswoman, non-edible shrimp shells are also often dumped into the sea, which may contribute to marine pollution. Similarly, snail shells, once the meat is removed, are frequently discarded into the environment, leading to land pollution. **Figure 1** illustrates piles of shrimp waste at the fishing port of Cotonou and snail shells in Allada (southern Benin).

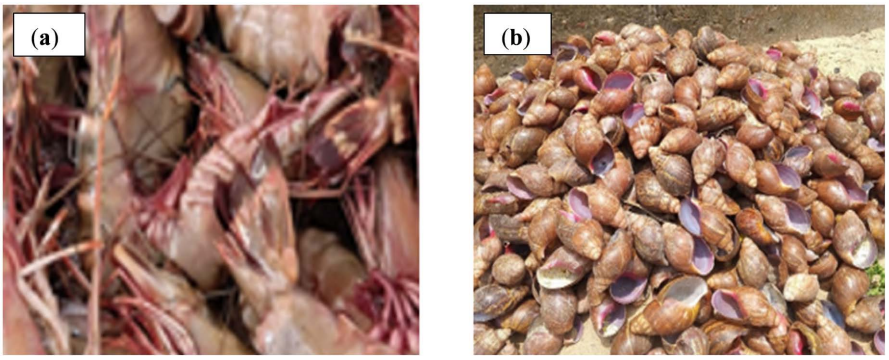

Figure 1. (a) Piles of shrimp waste at the fishing port of Cotonou; (b) Piles of snail shells in Allada.

This highlights the importance of developing value-added applications for these biopolymer-rich waste through sustainable extraction and utilization processes. The valorization of crustacean shell waste contributes significantly to environmental protection, particularly in coastal regions where seafood represents a major dietary component such as the fishing port of Cotonou. In such contexts, the sustainable recovery and utilization of these by-products is both necessary and highly valuable. In recent years, substantial progress has been made in chemistry of chitin and its derivatives [7] [8]. Chitin and chitosan have demonstrated essential functionality across a wide range of scientific and industrial fields [9]. It is estimated that the number of potential and established applications of chitin, chitosan, and their derivatives exceeds 200 distinct areas [10], encompassing sectors such as biomedicine, agriculture, food packaging and environmental remediation [11]-[13]. Chitin and chitosan are biodegradable, biocompatible and non-toxic biopolymers, properties that have supported their widespread application in the

development of films, hydrogels and semi-permeable membranes, particularly within the food industry and biomedical fields [14] [15]. On a global scale, chitin is recognized as the second most abundant natural polysaccharide after cellulose [16]. Structurally, it is composed of β (1→4)-linked 2-acetamido-2-deoxy-D-glucose units (N-acetylglucosamine) [17]. Chitin is the main organic component found in various organisms such as crustaceans, insects, fungi, and mollusks, particularly in the exoskeletons of crabs, shrimps, and related species. These biological materials have long served as abundant raw sources for chitin extraction. Chitin is regarded as a cellulose, in which the hydroxyl group at the C-2 position is substituted by an acetamido group [18]. When chitin undergoes deacetylation, the removal of its acetyl groups is converted into chitosan [19]. Chitosan (2-acetamide-deoxy-α-D-glucose) contains free amine groups that not only confer its polymeric nature but also render it a polycationic biopolymer. These characteristics make chitosan a promising material for the development of proton exchange polymer membranes. Structurally, chitosan is homopolymer derived from chitin, which is composed of β-(1→4) linked N-acetyl-D-glucosamine units [20]. The chemical structures of cellulose, chitin and chitosan are presented in **Figure 2**.

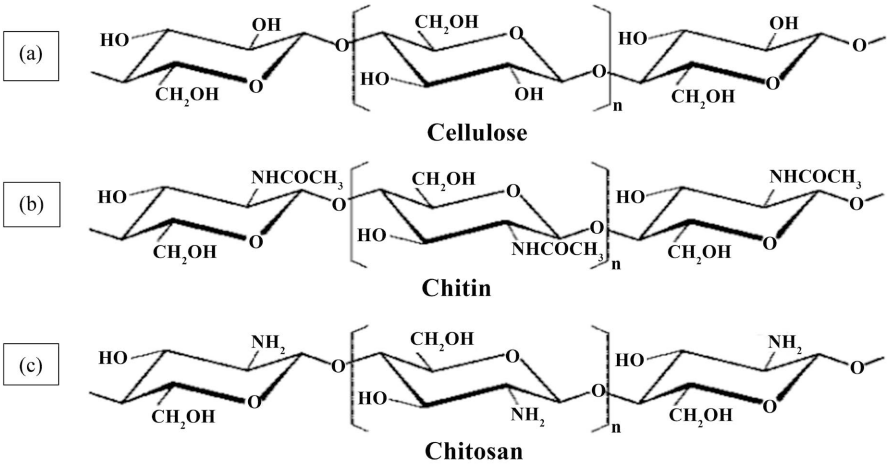

Figure 2. Structure of: (a) cellulose, (b) chitin, and (c) chitosan [20].

Unlike plant-based fibers, chitosan exhibits unique properties, including excellent film-forming ability and distinctive optical structural characteristics [21]. Moreover, it has positively charged compounds such as fats, lipids, and bile acids [22]. In recent years, chitin-derived polymers, particularly chitosan, have attracted significant attention as renewable polymeric materials for the development of proton exchange membranes. The performance of chitosan-based materials is typically assessed based on several parameters, including degree of deacetylation, solubility, and other physicochemical properties [23]. Hassainia *et al.* (2018) [24] demonstrated that in chitosan polymers, the percentage of glucosamine units, referred to as the degree of deacetylation (DDA), can range from 50% to 100%. Accurate knowledge of a chitosan sample's DDA is essential for assessing its physical

and biological properties. Consequently, the development of reliable and rapid methods for determining this parameter is of great importance. Over the past three decades, a variety of analytical techniques have been developed to measure DDA, including Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance spectroscopy (NMR), acid-base titration, potentiometric methods, and Gravimetric analysis [25]. Among these methods, acid-base titration stands out as the simplest, most practical, reproducible, and cost-effective technique that can be performed using standard laboratory equipment [26]. The aim of this study is to perform a comparative analysis of selected physicochemical properties of chitosan extracted from local sources versus commercial-grade chitosan. These findings highlight the potential of sourced chitosan as a sustainable raw material for the development of proton exchange membrane fuel cells, while also addressing a growing environmental issue in southern Benin.

2. Materials and Methods

2.1. Powder Sample and Chemicals Used

The shrimp shells were sourced from the fishing port of Cotonou, the country's largest seafood market. Upon arrival at the laboratory, the snail shells were washed, dried and ground into a fine powder. The frozen shrimp were purchased from a vendor and transported to the Laboratory. The edible shrimp meat was separated from the waste shells, which were then thoroughly washed. The cleaned shrimp shells were left to dry at room temperature on a laboratory bench for one week. Following this drying period, the shells were crushed using a metal mortar to obtain a powdered sample suitable for further processing (**Figure 3**).

In addition to the powdered samples, two keys' chemicals were used in this study. These are listed in the table below. Reactifs were obtained from AnalaR NORMAPUR and were used without any other purification (**Table 1**).

Commercial chitosan was also used in this study to compare the analytical results with those obtained from the chitosan produced in the laboratory. The commercial product, manufactured in China, was purchased from Thermo Scientific (batch number: A0440163). One of its key characteristics is its molecular weight, which ranges between 600 and 800 kDa.

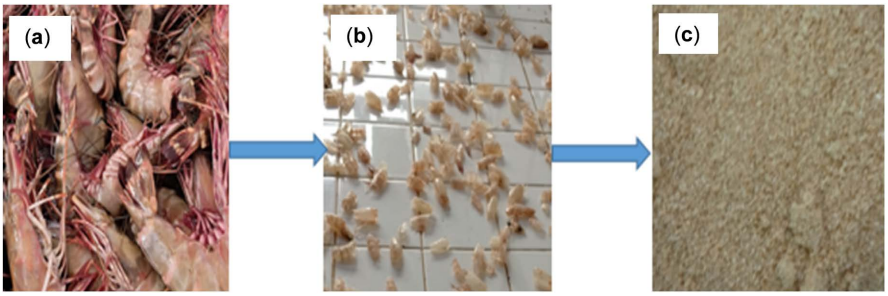

Figure 3. Sequential images showing the: (a) commercial shrimp, (b) dried shrimp shells after meat removal and washing, and (c) the final powdered form used for chitosan extraction.

Table 1. Chemical reagent used in the extraction of chitin and chitosan.

Chemical substance	Hydrochloric acid	Sodium hydroxide
Chemical formula	HCl	NaOH
Molecular weight (g/mol)	36.5	40
Density (kg/L)	1.16	2.13
Purity %	32	98.9

2.2. Experimental Protocol for Chitin and Chitosan Extraction

In accordance with the protocol employed by Zaghloul *et al.* (2019) [27], the subsequent protocol was implemented.

The powdered samples (snail shell powder and shrimp shell powder) were used for chitin extraction following a three-step process:

Deproteinization (Dp)

A 10% (w/v) sodium hydroxide (NaOH) solution was added to the powdered sample in a 250 mL round-bottom flask at a solid-to-liquid ratio of 1:30 (mv). The mixture was stirred using a magnetic stirrer and heated at room temperature for 3 hours. After cooling, the mixture was filtered under vacuum, and the solid residue was thoroughly washed with distilled water until neutral pH was achieved. The resulting materials were then dried in an oven at 60˚C for 24 hours.

Demineralization (Dm)

The solid obtained from deproteinization step was added to a 1 N hydrochloric acid (HCl) solution at a ratio of 1:20 (g/mL) in a 500 mL round-bottom flask. The mixture was stirred at room temperature for 3 hours using a magnetic stirrer. After the reaction, the solid residue was filtered under vacuum, thoroughly washed with distilled water until neutral pH was reached, and then dried in an oven at 60˚C for 24 hours.

Depigmentation (Dg)

A 0.315% sodium hypochlorite (NaOCl) solution was added to the demineralized powder at a ratio of 1:10 (g/mL) in a 250 mL round-bottom flask. The mixture was stirred using a magnetic stirrer for 1 hour at room temperature. It was then filtered and washed with distilled water until neutral pH was achieved. The resulting solid was dried in an oven at 60˚C for 24 hours. This step serves specifically to remove pigment compounds, primarily derivatives of β-carotene. The final product obtained is chitin, which can subsequently be used for chitosan production.

Chitosan was prepared from the extracted chitin by deacetylation using a 40% sodium hydroxide (NaOH) solution at a ratio of 1:20 (v/w). The mixture was stirred for 2 hours, then filtered, and the resulting solid was washed with distilled water until neutral pH (pH = 7) was achieved. The neutralized solid was dried in an oven, first at 60˚C, then at 100˚C for 3 hours. The resulting chitosan was stored for further analysis, alongside the chitin sample.

Before the analyses were conducted, an iodine water test was performed using commercial chitosan as a control sample.

The extraction yield of chitosan was calculated using the following formula [28]:

$$\text{Yield} = \frac{\text{mass of the chitin sample}}{\text{mass of chitosane sample}} \times 100 \quad (1)$$

2.3. Physicochemical Property Tests

Following the extraction of chitin and chitosan, various physicochemical parameters were investigated, with a particular focus on chitosan. These analyses aimed to evaluate the structural and functional quality of the extracted material in comparison with commercial chitosan.

Technique for Analyzing Samples Using X-Ray Diffraction

For this analysis, a Panalytical Empyrean Alpha 1 powder diffractometer equipped with a 45-sample changer was used to evaluate the crystallinity of the chitin and chitosan extracts. X-ray diffraction (XRD) enables the identification of crystalline phases, estimation of crystallite size, detection of structural defects, and assessment of other key structural features. The copper anode X-ray tube was used to generate Cu Kα radiation. During the measurement, the sample was rotated to maximize the number of crystallites brought into diffraction conditions. Diffraction patterns were recorded using a PIXcel 3D detector.

When a crystalline powder is irradiated with an X-ray beam of a specific wavelength, the electrons of the atoms scatter the X-rays. The scattered rays then interfere constructively or destructively, producing diffraction peaks at specific angles. The resulting spectrum, known as a diffractogram, displays the intensity of the diffracted beams as peaks at various 2θ angles.

This phenomenon is governed by Bragg's law, which relates the diffraction angle to the interplanar spacing d of the crystallographic planes. Bragg's law enables the calculation of these distances and, consequently, the determination of the crystal's lattice parameters. Each crystalline compound has a unique spectral signature, making phase identification possible.

Diffractogram analysis was performed using HighScore software, which compares the recorded pattern with reference data to identify the crystalline phases present in the sample. A sample that does not contain any crystals is considered amorphous. Such materials do not produce distinct diffraction peaks. Instead, their diffractograms typically display broad, unresolved bands, indicating the absence of long-range order and the presence of amorphous phases.

Thus, X-ray diffraction occurs only when the incident beam encounters a set of lattice planes (denoted by Miller indices (h, k, l)) at a specific angle, known as the Bragg angle, which satisfies the Bragg condition (**Figure 4**). Bragg's Law is expressed by the following equation [25]:

$$n\lambda = 2d \sin\theta \quad (2)$$

with

- n is the diffraction order (an integer, commonly 1);
- λ is the wavelength of the incident X-rays;

- d is the interplanar spacing, *i.e.*, the distance between adjacent crystallographic planes;
- θ is the Bragg angle or diffraction angle.

This fundamental equation describes the condition under which constructive interference of the reflected X-rays occurs, resulting in a measurable diffraction peak. It is essential for determining the crystallographic structure and lattice parameters of materials [20].

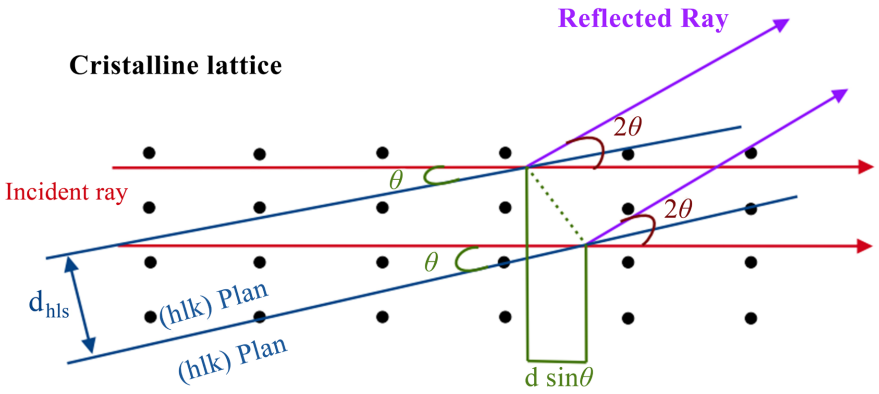

Figure 4. Schematic illustration of Bragg's Law principle. An incident X-ray beam interacts with parallel crystallographic planes in a crystalline lattice. Adapted from [24].

Melting Point of Extracted and Commercial Chitosan Samples

The thermal decomposition onset, corresponding to the melting point of the extracted and commercial chitosan sample, was determined using the Kofler WME system "Heizbank". This technique enables the observation of thermal transition by heating the samples on a calibrated temperature gradient. The melting point was identified as the temperature at which the sample began to visibly decompose or transition from solid to liquid phase under microscopic observation.

Degree of Deacetylation by pH Titration

The determination of the degree of deacetylation (DD) of both commercial and extracted chitosan samples was performed using an improved protocol based on the method described by Muzzarelli (1977) and Haidra (2018) [28] [29]. The experimental procedure was carried out in the following steps:

First, 0.1 g of chitosan was dissolved in a mixture of 10 mL of 0.1 M hydrochloric acid (HCl) and 10 mL of distilled water, and stirred for 30 minutes to ensure complete solubilization. Following this, an additional 12 mL of distilled water was added, and the mixture was stirred for another 30 minutes.

The resulting solution was then titrated, under continuous stirring, using a 0.1 M sodium hydroxide (NaOH) solution. During the titration, 0.5 mL increments of standard NaOH were added at regular intervals, and the pH was monitored every 2 minutes using pH indicator paper, with color changes recorded manually.

Each titration was performed in triplicate for both the commercial and extracted chitosan samples. The results were averaged to produce titration curves,

typically displaying two inflection points:

- The first inflection point (V_1) corresponds to the volume of NaOH required to neutralize the excess HCl.
- The second inflection (V_2) represents the volume needed to neutralize the total acidity of solution, including that contributed by the protonated amine groups of the chitosan.

The difference ($V_2 - V_1$) corresponds to the amount of NaOH required to deprotonate the amine groups, which directly relates to the degree of deacetylation of the chitosan. The DD% was calculated using the following equation, as reported by Haidra (2018) [29]:

$$\mathrm{DDA} = 2.03\frac{V_2 - V_1}{m + 0.0042\left(V_2 - V_1\right)} \tag{3}$$

with

- V_1 = volume of NaOH (in mL) required to neutralize the excess HCl;
- V_2 = volume of NaOH (in mL) corresponding to the complete neutralization of the solution;
- m = mass of solubilized chitosan (in grams), set at 0.1 g in this study.

This formula allows for the quantification of the degree of deacetylation based on the titration data, reflecting the proportion of deacetylated amino groups in the chitosan structure.

3. Results and Discussion

3.1. Chitosan Samples

The first set of results obtained in this study concerns the successful production of chitosan from chitin, following the outlined experimental protocol. Visual comparison between the chitosans is shown in the figure below.

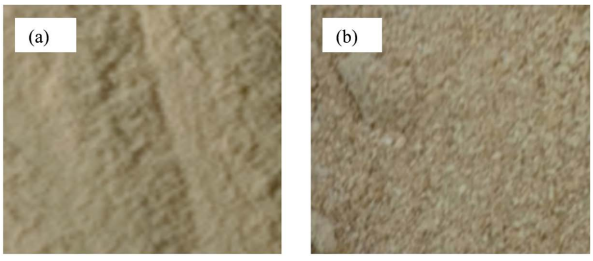

Figure 5. Photographs of chitosan samples: (a) commercial chitosan and (b) chitosan extracted from shrimp.

Figure 5 shows two types of chitosan: commercial chitosan and chitosan extracted from shrimp shells. The difference between these samples lies in their color, a key macroscopic parameter that attests above all to the quality, but also to the purity of the sample. Commercial chitosan (a) appears as a fine powder that is off-white/very light beige in color, indicating the high purity and quality of the sample. Chitosan extracted from shrimp shells (b) appears as a pale pink powder,

a well-known characteristic of chitosan derived from crustaceans. This color is more similar to that of commercial chitosan, but also to that of the sample obtained by Haidra (2018) [29].

After obtaining chitosan samples from the selected raw materials, X-ray diffraction (XRD) analysis was performed to characterize and verify the structural properties of the resulting product.

3.2. Analysis of Samples Using X-Ray Diffraction

Figure 6 shows the X-ray diffraction (XRD) patterns of chitosan powder extracted from shrimp shells and snail shells, illustrating the variation in peak intensity as a function of the diffraction angle (2θ).

This figure shows two distinct major peaks. The first peak, observed at approximately 20˚ (2θ), corresponds to the interatomic spacing of the (110) planes in the sample structure, characteristic of its semi-crystalline nature. This indicates, on the one hand, a partial organization of the polymer chains and, on the other hand, a moderate crystallinity typical of natural biopolymers. The second peak, located at approximately 10˚ (2θ), could suggest partial deacetylation during the experiment. These two peaks are approximately identical to those found by Mohanasrinivasan *et al.* and Ben Seghir *et al.* [30] [31].

The secondary peaks observed after 20˚ could correspond to impurities or other crystallographic planes (hkl) specific to the elementary lattice of chitosan, which are characteristic of the polymorphism of chitosan.

In addition, the various peaks observed confirm the moderate crystallinity and purity of the sample obtained. However, these results show that chitosan samples have lower crystallinity due to intramolecular hydrogen bonds resulting from the deacetylation of chitin. According to diffractogram (PDF 00-065-0890), we can also see that as the 2theta angle increases, the interplanar distance decreases. These results are similar to those presented in the literature [32] [33].

Using commercial chitosan as a reference, it is also possible to evaluate the quality of chitosan extracted from shrimp shells.

In this context, the XRD spectrum of commercial chitosan was recorded and compared with that of the extracted sample. The figure below shows the superimposition of the two corresponding diffractograms.

The diffractogram obtained for commercial chitosan is identifiable as that of an amorphous compound. It shows an almost total absence of crystallinity compared to chitin. This indicates a high degree of deacetylation of the chitin used in its production. Indeed, its diffractogram shows the absence of the numerous, poorly resolved peaks for chitin located at diffraction angles 2theta between 9˚ - 10˚, 19˚ - 20˚, 23˚, 26˚, 35 - 36˚, and 38˚ - 39˚. The diffractogram of commercial chitosan shows a single fairly broad band located in the 2θ angle range [10˚ - 30˚] and a very low intensity peak located at a 2θ angle close to 29˚. The wider band (2theta between 10˚ and 30˚) discreetly shows the initial peak of chitin located around 2theta equal to 9˚ -10˚ and the one around 19˚, but which are shifted respectively

towards the 2theta diffraction angles equal to 11˚ and 20˚. Careful examination of the two diffractograms shows that the "extracted chitosan" still contains peaks characteristic of chitin or polyglucosamine.

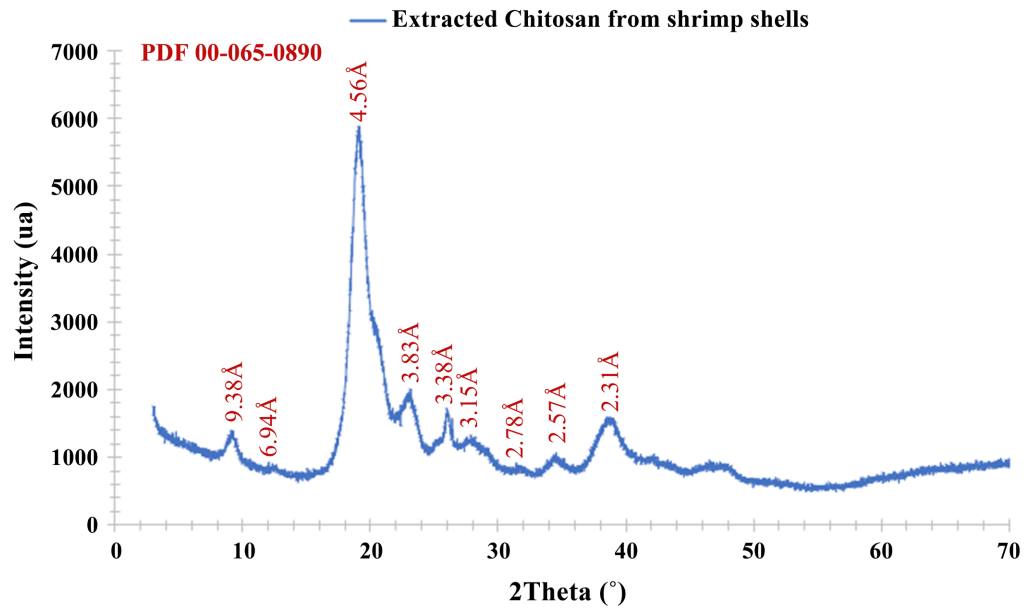

Figure 6. XRD diffractogram of chitosan extracted from shrimp shells.

This difference can be attributed to a lower degree of deacetylation in the extracted chitosan.

The XRD spectrum of chitosan (Figure 7) extracted from shrimp shells allows additional physicochemical properties to be evaluated in order to guarantee its purity.

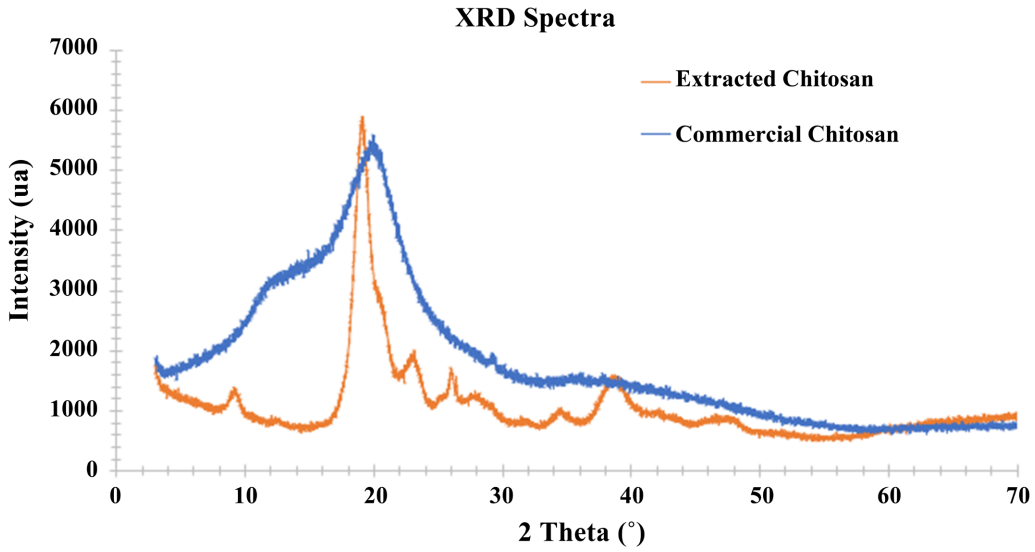

Figure 7. XRD spectra of commercial chitosan and chitosan extracted from shrimp shells.

3.3. Chitosan Yield

Determining the yield of chitosan extracted from shrimp shells is essential. The yield, calculated based on the assumption of a mole conversion between chitin and chitosan, is indicated in Table 2.

Table 2. Chitosan yield.

Sample	Chitin extracted (g)	Chitosan extracted (g)	Yield (%)
Mass	0.24	0.08	33.33

Analysis of the obtained value shows that the yield is relatively low, which could be attributed to losses caused by degradation of polymer chains. This result is relatively consistent with the work of Liyanage *et al.* 2022 [34], who reported a chitosan yield of 33.53% when investigating the potential of white shrimp shell waste as a source of chitosan in Sri Lanka.

3.4. Melting Point

The onset of thermal decomposition (melting point) of the different samples is presented in Table 3. Analysis of these values shows that the onset of decomposition for commercial chitosan is approximately 220˚C, while that of the chitosan extracted from shrimp shell is 235˚C. This difference may be attributed to the lower degree of deacetylation of the extracted chitosan, as previously suggested by the yield analysis.

Table 3. Melting points of extracted and commercial chitosan samples.

Samples	Melting point (˚C)
Chitosan extracted from shrimp shells	~235
Commercial chitosan	~220

3.5. Degree of Deacetylation by Potentiometric Titration

Figure 8 presents the pH titration curve of the commercial chitosan characterized in this study. The initial region of volume variation (0 - 3.5 mL) corresponds to the neutralization of free protons, with an initial pH of 2.0. Analysis of the curve reveals two distinct inflection points. The first inflection point, located at (V_1 = 3.5 mL, pH_1 = 2.3) corresponds to the deprotonation of the $-NH_3^+$ amine groups. The second inflection point, located at (V_2 = 9.5 mL, pH_2 = 11.5), corresponds either to the neutralization of hydroxyl groups (OH^-) or the completion of amine group neutralization. The intermediate region, spanning from 3.5 to 9.5 mL, represents the buffer zone associated with the $-NH_3^+/-NH_2$ equilibrium (Table 4).

The titration curve also displays a correlation coefficient of 0.94, indicating a strong fit and validating the accuracy of the identified inflection points. These results confirm the quality and reliability of the commercial chitosan sample, which can therefore be used as a reference for further samples.

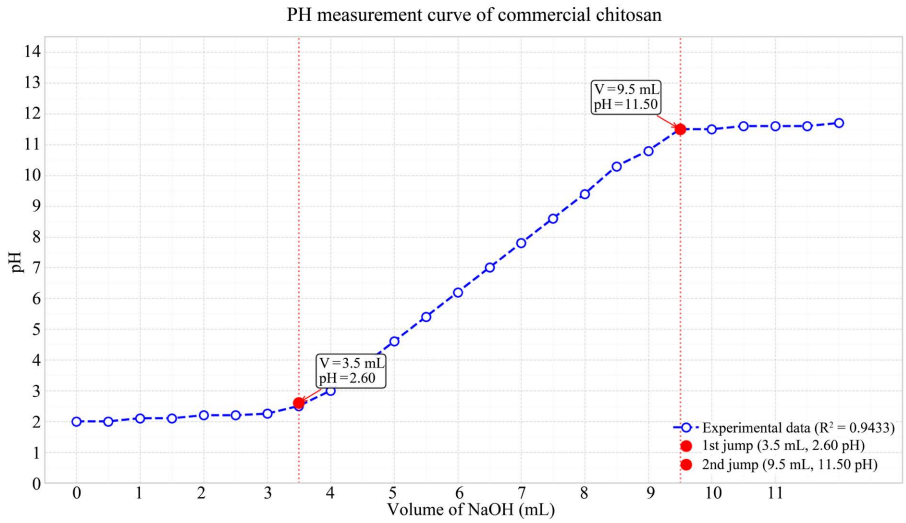

Figure 8. pH titration curve of commercial chitosan.

Figure 9 (below) presents the pH titration curve of chitosan extracted from shrimp shells in the laboratory. As with the commercial chitosan, two notable inflection points are observed. The first inflection point, located at (V_1 = 4.5 mL, pH_1 = 2.5), corresponds to the neutralization of the amine groups ($-NH_3^+$), while the second inflection point, at (V_2 = 7.5 mL, pH_2 = 10.0), corresponds to the titration of the hydroxyl groups (−OH). The high correlation coefficient obtained further supports the reliability and accuracy of the experimental data.

The two inflection points identified in the titration curves were used to calculate the degree of deacetylation for each chitosan sample. The results are summarized in **Table 4** below:

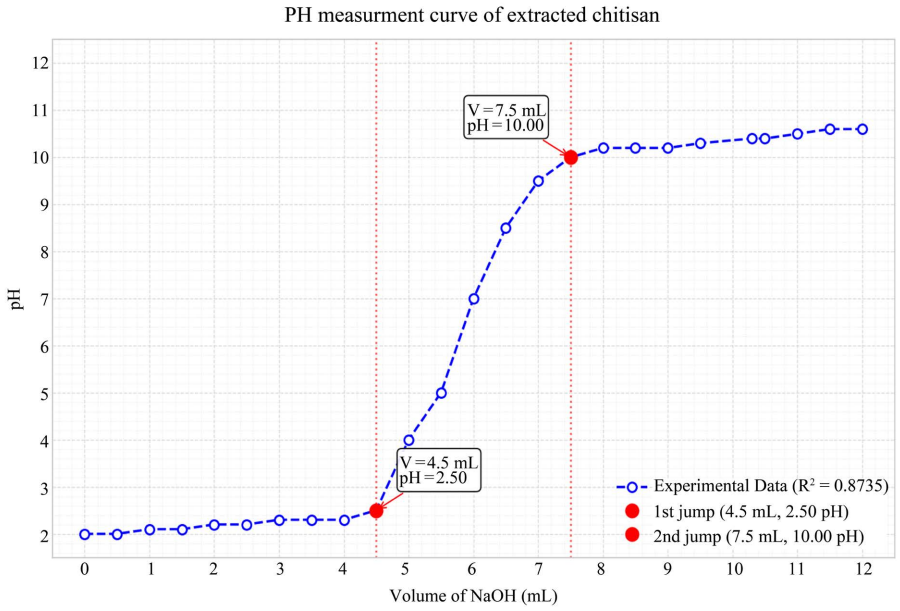

Figure 9. pH titration curve of chitosan extracted from shrimp shells in the laboratory.

Table 4. Degree of Deacetylation (DDA) of extracted and commercial chitosan samples.

Samples	Volume V_1 (mL)	Volume V_2 (mL)	Degree of deacetylation (DDA)%
Commercial chitosan	3.5	9.5	97.28
Extract chitosan	4.5	7.5	54.08

These results highlight a high degree of deacetylation of the commercial chitosan, confirming its purity and quality. In contrast, the extracted chitosan exhibits a significantly lower DD, which is consistent with previous observations regarding its yield and crystallinity, suggesting incomplete deacetylation during the extraction process.

4. Conclusion

Chitosan is one of the most valuable biopolymers due to its polysaccharide properties, including its biocompatibility, biodegradability, and non-toxicity. It is widely used in various fields, particularly in the development of proton exchange membranes. This study aimed to extract chitosan from locally available shrimp shells and compare their physicochemical properties with those of commercial chitosan. This will enable us to identify the most suitable local resource for PEM membrane synthesis. The main results revealed a chitosan yield of 33.33% from shrimp shells through the deacetylation of chitin. X-ray diffraction analysis confirmed that commercial chitosan is mainly amorphous, exhibiting a single peak of low intensity. In contrast, the extracted chitosan showed several peaks, suggesting a higher degree of structural organization. These differences are likely due to variations in the degree of deacetylation and processing conditions. A DDA of 54.08% was obtained for chitosan extracted from shrimp shells, compared to 97.28% for commercial chitosan.

According to the experimental protocol, chitosan was successfully extracted from shrimp shells and demonstrated structural and physicochemical characteristics comparable to those of commercial chitosan. The extracted chitosan is less pure but retains key properties that make it a candidate for applications such as the development of proton exchange membrane fuel cells (PEMFC). Future work will focus on improving the degree of deacetylation.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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