

Chemical Extraction of Chitosan from Shrimp Shells

Subjects: Fisheries

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The extraction of chitin and chitosan from raw shrimp wastes through three major processes: demineralization, deproteinization, and deacetylation. Chitin can be recovered from shrimp shells by removing minerals and proteins under diluted acidic and alkaline treatment, followed by a concentrated alkaline treatment to remove acetyl groups and obtain chitosan.

Keywords: shrimp shells ; chitosan ; chemical extraction

1. Chemical Demineralization of Shrimp Shells

Demineralization is the process of removing minerals, especially calcium carbonate. It could be conducted under the effect of inorganic or organic acids [1][2]. Therefore, demineralization is one of the most important stages in producing chitin and chitosan. It is usually obtained by inorganic acidic treatment. Shrimp shells have large amounts of minerals combined with proteins, chitin, and the rest of the exoskeleton. Calcium carbonate and calcium phosphate are the main minerals in the shells, which must be discarded to demineralize shrimp shells [3]. Most research studies reported that the demineralization process is preferred under the effect of diluted hydrochloric acid. Diluted hydrochloric acid can demineralize the shells by converting carbonate salts into chloride salts and carbon dioxide. Some studies performed the demineralization of shrimp shells by using organic acids, such as acetic and citric acids, through single or double demineralization steps [4][5][6]. The mineralization degree of shrimp shells, acid concentration, extraction temperature, and time are the main parameters affecting the efficiency of demineralization. Acid concentration is the most essential factor for controlling the removal of minerals. The pH neutralization of demineralized shells is crucial to stop the demineralization reaction. The quality of chitin production depends on the efficiency of the demineralization process, as the lower the mineral content, the higher quality of chitosan [4]. The demineralization of shrimp shells has been reported in recent literature under acidic treatment, ranging from a 1% to a 50% acid concentration for 1–24 h at 22–90 °C [1][2][3][4][5][6][7][8][9][10][11][12][13][14][15][16][17][18][19][20][21][22][23][24][25][26][27][28][29][30][31][32][33][34][35][36][37][38][39][40][41][42][43][44][45][46][47][48][49][50][51][52][53][54][55][56][57][58][59][60][61][62][63][64][65].

2. Chemical Deproteinization of Demineralized Shells

Chemical deproteinization is the process in which the chemical bonds between chitin and proteins are disrupted, and the biopolymer is depolymerized under the effects of an alkaline solution. Chemical deproteinization is an essential stage for removing the proteins from shrimp shells. Chemical deproteinization can be carried out either after or before the demineralization stage. Although deproteinization can be conducted under the effects of different alkaline solutions or chemical reagents, sodium hydroxide is the most preferred alkaline solution to use to disrupt the bonds between the chitin and proteins for protein removal or biopolymer hydrolysis. Non-optimized conditions for deproteinization could lead to a partial deacetylation of chitin. Alkali concentration, reaction temperature, and recovery time are the main drivers of the deproteinization process. The incomplete removal of protein affects the quality of chitin and chitosan and restricts their biomedical and pharmaceutical applications [4][34]. The conditions of the deproteinization of shrimp waste have been reported in recent studies under alkali concentrations ranging from 1% to 10% for 1–24 h at 22–100 °C [1][2][3][4][5][6][7][8][9][10][11][12][13][14][15][16][17][18][19][20][21][22][23][24][25][26][27][28][29][30][31][32][33][34][35][36][37][38][39][40][41][42][43][44][45][46][47][48][49][50][51][52][53][54][55][56][57][58][59][60][61][62][63][64][65].

3. Chemical Deacetylation of Chitin

Chitosan can be yielded by the chemical deacetylation of chitin, whereas the -NH₂ group replaces the acetyl group of C2 glucosamine [35]. The degree of acetylation is the differentiation factor between chitin and chitosan. The Deacetylation of chitin can be achieved using either acidic or alkaline solutions. However, deacetylation by the acidic medium is not the preferred option because it damages the glycosidic bonds and breaks the polymer chain. On the other hand, the concentrated alkaline deacetylation of chitin is a more efficient process for removing acetyl groups. Most of the literature reported conducting the deacetylation of chitin with a concentrated sodium hydroxide solution. The quality of the chitosan

is directly proportional to the degree of deacetylation, which depends on the alkali concentration, source of chitin, temperature, and time of chemical deacetylation [4]. The deacetylation conditions of chitin have been reported in recent researches using concentrated sodium hydroxide using concentrations ranging from 30% to 65% for 40 min to 72 h at 22–100 °C [1][2][3][4][5][6][7][8][9][10][11][12][13][14][15][16][17][18][19][20][21][22][23][24][25][26][27][28][29][30][31][32][33][34][35][36][37][38][39][40][41][42][43][44][45][46][47][48][49][50][51][52][53][54][55][56][57][58][59][60][61][62][63][64][65].

4. The Influence of Chemical Extraction Stages on Chitin and Chitosan Yield Percentage

Different authors have reported chemical demineralization, deproteinization, and deacetylation under various conditions of temperature, time, and acid and alkali concentrations. The variation of demineralization and deproteinization conditions in different articles resulted in different chitin and chitosan yields, even if some experimental studies did not report the chitin and chitosan yield percentages in their results [7][17][21][36].

Research on the black tiger shrimp (*Penaeus monodon*) was carried out to obtain chitin and chitosan from shrimp co-products (shells). Two different methods were applied in this study to extract chitin and chitosan from the shrimp co-products. In the first method, different treatments of 50 mL HCl were applied (3%, 4%, and 5%) at room temperature with continuous stirring at 150 rpm for 90–120 min, while deproteinization was conducted using 6% NaOH for 60 min at room temperature to produce chitin percentages 23.43 ± 0.21 , 22.70 ± 0.20 , and 22.13 ± 0.15 . Chemical deacetylation to recover chitosan from chitin was applied by the suspension of 1 g of each chitin sample in 50 mL of 50% sodium hydroxide solution at room temperature which was stirred at 150 rpm for 90 min. The solids were filtered off using a 250-micron sieve with deionized water until neutralization, then dried at 70 °C to obtain 21.44 ± 0.19 , 20.75 ± 0.12 , and 20.07 ± 0.15 percentages of chitosan yield. In the second method, the same concentrations of 50 mL HCl were used (3%, 4%, and 5%) under heating of 60 °C with continuous stirring at 150 rpm in a water bath for 90–120 min., while the deproteinization was performed under 5% NaOH for 60 min in a water bath under heating of 60 °C with constant stirring at 150 rpm to obtain 25.70 ± 0.30 , 25.33 ± 0.20 , and 24.37 ± 0.30 percentages of chitin. Then, the chitin was deacetylated under the same conditions as the first method, except the heating was at 60 °C to obtain chitosan yield percentages of 23.24 ± 0.24 , 22.97 ± 0.15 , and 22.40 ± 0.26 [16].

A research study has applied chemical extraction using three levels of diluted HCl (2%, 3%, and 4%) at ambient temperature (28 ± 2 °C) to demineralize the shrimp waste for 16 h, while chemical deproteinization was conducted under a unique concentration of 4% sodium hydroxide for 20 h to produce three different yields of chitin at 17.36%, 14.02%, and 13.12%. The deacetylation of chitin was applied by using four different concentrations (30%, 40%, 50%, and 60%) of NaOH at 65 °C for 20 h to obtain a 15.4% of chitosan yield [37].

In contrast, another researcher performed the chemical deproteinization before the demineralization as an initial phase. The deproteinization was conducted with 3.5% NaOH at 90 °C with continuous stirring for 1 h, followed by chemical demineralization under the effect of 6% HCl at 90 °C for 1 h; the obtained chitin yield was 40%. Then, chitin was converted to chitosan under the effect of 50% NaOH at various heating temperatures and times (70, 80, 90, and 100 °C for 40, 60, and 80 min, respectively) to obtain higher yield percentages of chitosan at 83.74, 72.17, 61, and 54.66 [38].

An optimization study applied the chemical method to optimize the recovery of chitin and chitosan from shrimp shells at ambient temperature under different concentrations of acids and bases for demineralization and deproteinization, respectively. Five treatments of hydrochloric acid (10%, 20%, 30%, 40%, and 50%) were applied to chemically demineralize the shrimp shells to produce five samples of demineralized shrimp shells. For each demineralized sample, the chemical deproteinization was conducted using four levels of sodium hydroxide (1.5%, 3%, 6%, and 8%) to obtain twenty yields of extracted chitin with different yield percentages. Then, the chitin yields were treated overnight with 50% sodium hydroxide at 60–70 °C heating temperature for the chitin deacetylation to produce the various ranges of chitosan yield percentages (Table 1). The optimization process found that the optimal concentration of hydrochloric acid and sodium hydroxide to produce good quality white chitin and chitosan yield with lower acidic and alkaline residual impact on the environment was 30% and 6%, respectively [39].

Table 1. Chitin and chitosan yield percentage under distinctive chemical extraction conditions [39].

Demineralization Acid Concentration (%)	Deproteinization Alkali Concentration (%)	Chitin Yield %	Deacetylation Alkali Concentration (%)	Deacetylation Temperature (°C)	Chitosan Yield %
10	1.5	30.6	50	60–70	30.00

Demineralization Acid Concentration (%)	Deproteinization Alkali Concentration (%)	Chitin Yield %	Deacetylation Alkali Concentration (%)	Deacetylation Temperature (°C)	Chitosan Yield %
10	3	29.1	50	60–70	28.00
10	6	28.7	50	60–70	27.00
10	8	27.7	50	60–70	25.20
20	1.5	28	50	60–70	27.80
20	3	27.7	50	60–70	27.50
20	6	26.4	50	60–70	25.80
20	8	24.9	50	60–70	24.30
30	1.5	24.4	50	60–70	24.10
30	3	24	50	60–70	23.80
30	6	22.6	50	60–70	21.76
30	8	21.8	50	60–70	20.50
40	1.5	23.8	50	60–70	22.80
40	3	22.6	50	60–70	22.20
40	6	21.1	50	60–70	20.60
40	8	20.2	50	60–70	19.50
50	1.5	22.1	50	60–70	20.90
50	3	20.7	50	60–70	19.50
50	6	19.4	50	60–70	17.80
50	8	18.2	50	60–70	15.40

Another technique of the chemical extraction of chitin and chitosan was conducted by boiling the collected shrimps in salty water for 10 min, peeling them with an automated machine and separating the shells from the meat, drying them at 50 °C in a dry oven for 24 h, grinding them in a laboratory mixer, and storing them at –25 °C for further recovery processing. The first phase of the recovery process of chitin and chitosan from shrimp shells was the deproteinization, conducted by adding 30 mL of 2% NaOH solution to 1 g of shells at 90 °C for 2 h, and then centrifuging them at 4000 rpm for 15 min to separate the alkali-insoluble fraction. Then, a reflux of 10% v/v acetic was applied at 60 °C for 6 h. Then, 10.8% chitin was separated under the effect of centrifugal power at 4000 rpm for 15 min, and 8.2% of chitosan was yielded under the effect of 8% NaOH solution at pH = 9 ^[40].

Other researchers have prepared shrimp shells in three different particle sizes, 16, 32, and 60 mesh knit, to obtain chitin and chitosan. Chemical demineralization was conducted using 2% HCl at 30 °C for 12 h. This was followed by separating the alkali-insoluble fraction by centrifugation at 4000 g for 15 min. Then, the precipitate was neutralized with distilled water. Chemical deproteinization was applied under the effect of 4% NaOH alkaline solution at 90 °C for 12 h to obtain three diverse levels of chitin: 79.4%, 74%, and 42.2% for 16, 32, and 60, respectively. While the deacetylation of the three different chitin samples has been conducted with a new technique under the effect of 45% NaOH in a microwave oven for irradiation at 600 watts using two methods. The first method was continuous irradiation for 15 min to obtain 44.8%, 52.2%, and 44.4% yields of chitosan. The second method applied pulsed irradiation six times, with stirring after 5 min of each irradiation pulse to produce 43%, 32.2%, and 42.4% yields of chitosan ^[41].

The sequence of demineralization and deproteinization has a significant impact on chitin and chitosan yield. reported that the sequence of demineralization and deproteinization stages has a direct impact on chitin and chitosan yield, whereas the beginning chemical extraction with demineralization followed by deproteinization is highly recommended ^{[34][42]}.

5. Characteristics of Chitosan Chemically Obtained from Shrimp Shells

Recent articles have discussed the synthesis of chitosan from shrimp shells and showed that chitosan has a variety of significant physicochemical properties and biological functionality ^[43], such as biodegradability, bioactivity, non-toxicity, active linear amino polysaccharide with a high nitrogen content with complexing and chelating ability, hydrophilicity,

hypolipidemic, water insolubility, solubility in diluted organic and inorganic acids, crystallinity, cross-linking and chemical activation through its reactive groups, ionic conductivity, polyelectrolyte properties in an acidic medium, salt formation with organic and inorganic acids, positively charged biopolymers acting as a flocculating agent regarding its ability to interact with negatively charged molecules, absorbency, adhesivity, biocompatibility, blood anticoagulant properties, antitumor and antimicrobial activities, film-forming ability, and high viscosity regarding its ability to form intermolecular hydrogen bonds [44][45].

The influence of the DD (degree of deacetylation) and solubility degree are the main crucial factors in determining the quality of the physicochemical and biological characteristics of chitosan, depending on the nature, species, and the techniques used in the chitosan synthesis from chitin and the optimization of the extraction processes (demineralization, deproteinization, decolorization, and deacetylation) and their conditions (concentrations, temperature, and time). The DD (degree of deacetylation) is represented by the number of free amino groups ($-NH_2$) in chitosan as polysaccharides and can be used to indicate the difference between chitin and chitosan through the number of acetyl groups removed from chitin to form chitosan. The removal of acetyl groups affects the physicochemical properties of chitin and chitosan and their appropriate applications in different fields [36][46][47]. The testing of chitosan characteristics, according to standard methods, is very important in order to confirm chitosan synthesis, purity, and yield percentage. In most of the previous studies, chitosan physicochemical properties were analyzed by using various analytical techniques to ensure the quality of chitosan by the determination of the following parameters [48].

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