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DEVELOPMENT OF TECHNOLOGY OF PRODUCTION OF CHITIN FROM CRUSTACEANS WASTES

Abstract. The major sources of chitin production are sea crustaceans – shrimps and crabs. In Egypt, the most economically justified source of chitin is green shrimp *Penaeus semisulcatus*, despite the fact that it can cause a significant pollution of the sea aquatorium. The aim of the study is to analyse the chemical composition of the crude shell of green shrimp and to develop the technology of producing chitin from this raw material. It has been found that the green shrimp shell contains 44.96% of alkali, 36.63% of protein, 4.85% of fat, 7.38% of carbohydrates and 6.18% of fiber. The moisture is 13.05%. While producing chitosan (chitin), the effect of the concentration of chlorohydric acid, temperature and time on the content of alkali is studied. It has been stated that while producing chitosan (chitin), the optimal concentration of HCl, at which the quantity of alkali is reduced by 91.98%, is 2M. This result is obtained at thermostating for 2 hours at the temperature 45°C. The best result of deproteinization is received when using 1M NaOH at the temperature 75°C for 4 hours.

Key words: shrimps, chitin, chemical composition, demineralization, deproteinization.

Introduction

Huge amounts of crab and shrimp shells have been abandoned as wastes by worldwide seafood companies. This has led to considerable scientific and technological interest in chitin as an attempt to use these renewable wastes [1]. Chitin, a naturally abundant mucopolysaccharide, and the supporting material of invertebrates such as crustaceans, insects, consist of a homopolymer of β-(1→4) linked 2-acetamido-2-deoxy-d-glucopyranose residues. In spite of the presence of nitrogen, it may be regarded as cellulose with hydroxyl at position C-2 replaced by an acetamido group. Like cellulose, it functions as structural polysaccharides. Its natural production is inexhaustible; arthropods, by themselves, count more than 106 species from the 1.2 × 106 of total species compiled for the animal kingdom, constitute permanent and large biomass source. Chitin is a white, hard, inelastic, nitrogenous polysaccharide and the major source of surface pollution in coastal areas.

About 45% of processed seafood consists of shrimp, the worst of which is composed of exoskeleton and cephalothoraxes [2, 3]. The latter has become a problem for the environment. This waste represents 50–70% of the weight of the raw material; however, it contains valuable components such as protein and chitin [4]. Chitin, next to cellulose, is the second most common polysaccharide on earth, with a yearly production of approximately 1010–1012 tons [5]. About 40.000 tons were caught globally and 34.050 tons – from the North Sea in 2007. Approximately 70% of the total shrimp landing becomes wastes, so a tremendous tonnage of shrimp wastes is being produced. The wastes from shrimp processing mainly consist of heads, shells and residual meat, which is unsuitable for human food production. A large portion of the wastes is chitin, which is not readily digestible and thus the wastes cannot be used commercially as animal feed [6, 7].

Our objective is to appraise the state of the art concerning this polysaccharide: its morphology in the native solid state, methods of identification and characterization and chemical modifications, as well as the difficulties in utilizing and processing it for the selected applications [8, 9]. Approximately 70% of the landed value of shellfish is rejected as offal. This abundant waste material has either to be discarded or converted to value added products, and this has led to the production of several useful biochemicals and nutrients, such as chitin, pigments and seafood peptones from these by-products [10, 11]. Chitin is found in marine invertebrates, insects and fungi, as the conjugated form with proteins. It is frequently present as a cell wall material in plants, and in the cuticle of animals. In addition, chitins in animal tissues are frequently calcified, such as in the case of shellfish. Some fungi contain chitosan; however, it is commercially produced by the distillation of chitin [12, 13].Chitin is usually isolated from the exoskeletons of crustaceans, mollusks, insects and certain fungi. Three different polymorphs of chitin are found in nature; the α-chitin, being the most common structure and corresponding to tightly compacted orthorhombic cells formed by alternated sheets of antiparallel chains [14, 15], the β-chitin, adopts a monoclinic unit cell where the polysaccharide chains are disposed in parallel fashion and γ-chitin, however it has not been completely identified, an arrangement of two parallel and one antiparallel sheet has been proposed. It has been suggested [4] that chitin can be a combination of a and

b structures rather than as a different polymer. α-Chitin is usually isolated from the exoskeleton of crustaceans and more particularly from shrimps and crabs. β-Chitin can be obtained from squid pens, while γ-chitin exists in fungi and yeast. Because chitin has a compact structure, it is insoluble in most solvents. Therefore, the chemical modifications of chitin are performed [16, 17]. The most common derivative is chitosan, derived by partial deacetylation of chitin. When the degree of deacetylation (DDA) reaches higher than 50%, chitosan becomes soluble in acidic aqueous solutions and it behaves as a cationic polyelectrolyte.

Several techniques to extract chitin from different sources have been reported. The most common method is referred to as the chemical procedure. The chemical method for isolation of chitin from crustacean shell biomass involves various major steps: elimination of inorganic matter (calcium carbonate) in dilute acidic medium (demineralization), and usually demineralization is accomplished by using HCl. Followed by extraction of protein matter in alkaline medium (deproteinization), and it is traditionally done by treating shell wastes with aqueous solutions of NaOH or KOH. The effectiveness of alkali deproteinization depends on the process temperature, the alkali concentration, and the ratio of its solution to the shells. As an alternative to the chemical process, a biological process using microorganisms has been evaluated for the demineralization [18, 19] and the deproteinization [13]. Recovery of the protein fraction of the shrimp wastes by enzymatic hydrolysis has widely been investigated [15–20].

Chitin is usually isolated from the exoskeletons of crustaceans and more particularly from shrimps and crabs where α-chitin is produced [21]. Squid is another important source of chitin, in which it exists in the β form, which was found to be more amenable for deacetylation. It also shows higher solubility, higher reactivity and higher affinity towards solvents and swelling than α-chitin due to much weaker intermolecular hydrogen bonding ascribable to the parallel arrangement of the main chains [22, 23]. Many authors [24, 25] have tackled the problem of extracting chitin from its natural sources followed by its deacetylation to obtain the much more useful material chitosan. Potential and usual applications of chitin, chitosan and their derivatives are estimated to be more than 200 [26]. This wide range of applications includes cosmetics, agriculture, food, biomedical and textile, as chelating agents and refinement industrial effluents.

Material and methods

Shells of green shrimp *Penaeus semisulcatus* were purchased from *Abou Ghalli Company* for trading and exporting *Alabour* market, Egypt. The shells were manually scraped (free of loose tissue), collected and brought to the laboratory in the same day. Whenever, the shells were brought to the laboratory, they were frozen immediately (at –18°C) and stored for further analysis.

The shells were first washed several times with tap water and rinsed several times with distilled water. The rinsed shells were dehydrated in an electric draft oven at 45°C tall drying. The dried shells were grounded in a grinder (Braun Biotech International GMBH. D.34212 Melsungen, Germany) to pass through a 1.6 mm sieve and stored at 4ºC in tight dark glasses till it was subjected to demineralization and deproteinization process.

The procedure [27] was followed for the determination of moisture (method No. 32.1.03), crude fat (method No. 32.1.13), crude fiber (method No. 32.1.15), crude protein (method No. 32.1.22) and total ash (method No. 32.1.05). Total carbohydrate content was calculated by difference. A preliminary experiment to define the optimum demineralization condition of shrimp shells was carried out using 50g dried shrimp shells. The raw materials were obtained in solid form from the different sources, washed with water, desiccated at room temperature and cut into small pieces. Demineralization was carried out at the room temperature using 1M hydrochloric acid bathes. Demineralization was carried out using 1M HCl at 45°C for 2 hours with a solution/solid ratio of 1/15 v/w. The HCl, which showed the highest alkali reduction rate (2M) was applied during determination of the optimum temperature (45°C). The temperature (45°C) resulted in the highest alkali reduction rate was applied during determination of the optimum treatment time (2hr). The optimum conditions for demineralization treatment were 2M HCl at 45°C for 2hr.

A preliminary experiment to define the optimum conditions for deproteinization of demineralized shrimp shells was carried out using 50g demineralized dried shrimp shells. Deproteinization was carried out using NaOH concentrations (1M NaOH) at the temperature 75°C for 4 hours with a solution/solid ratio of 1/15 w/v. The NaOH, which showed the highest protein reduction rate (1M), was applied during determination of the optimum temperature (75°C). The temperature (75°C) showed the highest alkali reduction rate was applied during determination of the optimum treatment time (4hr).Statistical analysis was made using the analysis of variance (ANOVA), and least significant dif-

ference (LSD) was obtained to compare the means of treatments, using Costat version 6.311 (Copyright 1998–2005, CoHort Software). Duncan's multiple range test [28] was used to compare the treatment means.

Results and discussion

Proximate composition of crude shrimp shells (moisture, protein, fat, alkali, carbohydrates and fiber content), was presented in Table 1. The protein content was 36.63%, while alkali content was 44.96%.

Table 1

Chemical composition of crude shrimp shells (on dry weight basis)

Samples	% Moisture	% Protein	% Fat	% Carbohydrates	% Ash	% Fiber
Crud shrimp shells	13.05	36.63	4.85	7.38	44.96	6.18

Our results showed a low content of lipids (4.85%), while shrimp shells total fiber and total carbohydrate content were 6.18 and 7.38%, respectively. These results are in the same trend [8, 28, 29] which state that dry Crangon shells consist of 10–38% of proteins, 31–44% of minerals. While ours are close to that reported by [30]. The content of total fiber was 29.41% in chitin compared to 6.18% of shrimp shell wastes.

Effect of HCl concentration. Generally, using different HCl concentrations significantly ($p \leq 0.05$) decreased the alkali content compared with untreated crude shells Table 2. On the other side, no significant ($p > 0.05$) differences were noticed in alkali content among 1, 2 and 3M HCl (5.98, 3.62 and 6.06%, respectively). While treating crude shells with 2M HCl showed the highest alkali reduction rate (91.98%) compared with 1M, 3M, 4M and 5M (86.76, 86.58, 76.49 and 59.67%, respectively). A concentration of 2 M was used for further extraction treatment. These results are similar to those reported by [31, 32].

Table 2

Effect of different molarities of HCl on alkali content of shrimp shells (on dry weight basis)

HCl concentration (M)	% Total Alkali	% Reduction rate
Washed crud shells	44.96 [a]	–
1	5.98 d	86.76
2	3.62 d	91.98
3	6.06 d	86.58
4	10.62 c	76.49
5	18.22 b	59.67
L.S.D	3.76	–

[*] Means in the same column with different letters are significantly different at ($p \leq 0.05$).

Effect of temperature. The highest reduction rate of alkali content was obtained when crude shells treated at 45°C (98.2%) followed by that treated at 60°C (96.12%) compared to the other temperatures Table 3. No significant differences ($p > 0.05$) were noticed in alkali content between the shells treated with HCl at 45°C and that treated at 60°C (0.81 and 1.75% respectively). While both of them were significantly ($p \leq 0.05$) lower than the washed crude shells (44.96%).

Table 3

Effect of different temperature Degrees on alkali content of shrimp shells (on dry weight basis)

Temp (°C)	% Total Alkali	% Reduction rate
Washed crud shells	44.96 [a]	–
30	2.71 [bc]	94.00
45	0.81 [d]	98.2
60	1.75 [cd]	96.12
75	2.89 [b]	93.6
90	2.36 [bc]	93.5
L.S.D	1.07	–

[*] Means in the same column with different letters are significantly different at ($p \leq 0.05$).

Effect of time. Generally, using different extraction times significantly affect ($p \leq 0.05$) the alkali content compared with untreated crude shells (44.96%) (Table 4). On the other side, no significant ($p > 0.05$) differences were noticed among extraction for 2 hr, 3 hr and 4 hr (2.53, 3.89 and 3.22%, respectively).

Table 4

Effect of different extraction times on alkali content of shrimp shells (on dry weight basis)

Time (hr)	% Total Alkali	% Reduction rate
Washed crud shells	44.96 [a]	–
1	5.94 [c]	86.85
2	2.53 [d]	94.40
3	3.89 [cd]	91.38
4	3.22 [d]	92.87
5	9.57 [b]	78.71
L.S.D	2.12	–

[*] Means in the same column with different letters are significantly different at ($p \leq 0.05$).

Extraction for 2 hr showed the highest reduction rate (94.40%), followed by that treated for 4hr (92.87%), 3hr (91.38%) and finally that treated for 1 hr (86.85%). While, the lowest alkali reduction rate was obtained by extracting for 5hr (78.71%).

The results of Tables 2–4 indicated that the optimum conditions for reducing alkali content of shrimp shells were extracted with 2M HCl at 45°C for 2hr.

Deproteinization treatment (chitin production)

Effect of NaOH concentration. Table 5 concluded that using different NaOH concentrations significantly ($p \leq 0.05$) decreased the protein content compared with demineralized crude shells (9.60%). On the other side, no significant ($p > 0.05$) differences were observed in protein content of shrimp shells treated with 1M and 2M NaOH (3.44 and 3.63%, respectively), which showed the highest reduction rate in total protein (63.93 and 61.75%, respectively). While treating crude shrimp shells with 5M NaOH showed the lowest ($p \leq 0.05$) reduction rate of protein (28.06%).

Table 5

Effect of different concentrations of NaOH on protein content of demineralized shrimp shells

NaOH Concentration (M)	% Total protein	% Reduction rate
Crude Demineralized	9.60 [a]	–
1	3.44[e]	63.93
2	3.63 [de]	61.75
3	4.71 [cd]	56.49
4	4.59 [cd]	52.12
5	6.93 [b]	28.06
L.S.D	1.11	–

[*] Means in the same column with different letters are significantly different at ($p \leq 0.05$).

The previous results showed that 1M NaOH concentration was used for further deproteinization treatment. A complete protein removal from shrimp shells does not necessarily indicate a high quality chitin, as prolonged incubation times of shrimp shells at elevated temperatures in the concentrated alkali solution, on the one hand, remove protein effectively, but, on the other hand, may result in breakage of the N-acetyl Glucosamine polymer chains of chitin [32].

Extraction of protein matter in alkaline medium (deproteinization) is traditionally done by treating shell waste with aqueous solutions of NaOH or KOH. The effectiveness of alkali deproteinization depends on the temperature, the alkali concentration, and the ratio of its solution to the shells. In industrial scale it would not be economical anyway to first fully dry the shells and then add NaOH for protein hydrolysis, if hydrolysis could be achieved with moist shells and more concentrated NaOH as well. This is contrary to most of the optimization studies in the labs, which generally take dried starting material for chitin extraction [24–33].

Effect of temperature. The highest reduction rate of protein content was obtained when the process was carried out at 75°C (72.63%) compared to the other temperatures (Table 6). No significant differences ($p > 0.05$) in protein content were noticed between the shells treated with NaOH at 45°C and that treated at 60 °C (3.61 and 3.75%, respectively). It was reported that the optimum deproteinization was carried out at temperature ranged from 30 to 65°C. The temperature 75°C, which represents the highest protein reduction rates, were used for further deproteinization treatment.

Table 6

Effect of different temperature degrees on protein content of demeneralized shrimp shells

% Reduction rate	% Total protein	Temp (°C)
–	9.59 [a]	Crud Demineralized
32.03	6.52 [b]	30
62.28	3.61 [cd]	45
60.84	3.75 [cd]	60
72.63	2.62 [d]	75
65.21	3.34 [cd]	90
–	1.26	L.S.D

[*] Means in the same column with different letters are significantly different at ($p \leq 0.05$).

Effect of time protein content was affected ($p \leq 0.05$) by extraction time. No significant ($p > 0.05$) differences in total protein content were observed between 1hr and 2hr of extraction. Also, no significant ($p \leq 0.05$) differences in protein content were observed among 2, 3, 4 and 5 hr of extraction. However, the lowest reduction rate was obtained, when 1hr extraction was used (34.58%) (Table 7). The slight improvement in protein removal after 5hr (as compared to 4hr) was associated with a decrease of viscosity at both mesophilic and thermophilic temperatures [25]. Extraction is usually accomplished with a mild alkaline solution, such as 1 or 2% sodium hydroxide, at 60–70 °C, for a few hours, and the extracted proteins can be recovered for other uses [34].

Table 7

Effect of different time on protein content of demineralized shrimp shells

% Reduction rate	% Total protein	Time(hr)
–	9.59 [a]	Crud Demineralized
34.58	6.27 [b]	1
55.83	4.23 [c]	2
61.16	3.72 [cd]	3
68.40	3.03 [d]	4
61.69	3.67 [cd]	5
–	**1.05**	**L.S.D**

[*] Means in the same column with different letters are significantly different at ($p \leq 0.05$).

Deprotenization (≤ 5% residual protein in shrimp shells) is possible in combination of both mesophilic temperatures (30°C) and long incubation time (5hr) or at thermophilic temperatures (55 °C) and short incubation time (2hr) indicating that short incubation times are preferred thermophilic temperatures must be applied. However, protein and mineral content are not the only parameters to be considered for chitin quality [21]. It was stated that when the alkali treatment for deprotenization of shrimp shells was extended over more than 4hr [30–35], at all incubation temperatures a decrease of chitin viscosity was observed. The slight improvement in protein removal after 5hr (as compared to 4hr) was associated with a decrease of viscosity at both mesophilic and thermophilic temperatures. Chitin viscosity was increased with increasing deproteinization temperatures from 30 to 55 °C, but it was decreased at higher temperatures, e. g. at 65°C.

The results (Tables 5–7) indicated that the optimum conditions for deprotenization treatment of shrimp shells were 1M NaOH at 75°C for 4hr.

Conclusion

Chitin has been isolated from local marine sources, by treatment with dilute HCl solution for demineralization and dilute NaOH for deproteinization. The best condition for shrimp shell demineralization process was 2M HCL at 45ºC for 2hr. Meanwhile, the best condition to remove the protein from shrimp shells was 1M NaOH at 75ºC for 4hr. Meanwhile, the best condition for the production of shrimp shell chitosan was 10M NaOH at 90ºC for 2hr.

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РАЗРАБОТКА ТЕХНОЛОГИИ ПРОИЗВОДСТВЕ ХИТИНА ИЗ ОТХОДОВ РАКООБРАЗНЫХ

Основными источниками получения хитина являются морские ракообразные – креветки и крабы. В условиях Египта наиболее экономически обоснованным источником хитина может стать зелёная креветка *Penaeus semisulcatus*, несмотря на то, что способна вызвать значительное загрязнение морской акватории. Цель исследования – анализ химического состава сырого панциря зелёной креветки и разработка технологии получения хитина из этого сырья. Выявлено, что панцирь зелёной креветки содержит 44,96 % золы, 36,63 % белка, 4,85 % жира, 7,38 % углеводов и 6,18 % волокон. Влажность составила 13,05 %. В ходе получения хитозана (хитина) исследовалось влияние конентрации соляной кислоты, температуры

и времени на содержание золы. Установлено, что при получении хитозана (хитина) оптимальная концентрация HCl, при которой количество золы снижалось на 91,98 %, составила 2 М. Этот результат был достигнут при термостатировании в течение 2 часов при температуре 45 °С. Наилучший результат депротеинизации был получен при использовании 1 М NaOH при температуре 75 °С в течение 4 часов.

Ключевые слова: креветки, хитин, химический состав, деминерализация, депротеинизация.

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