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OPTIMIZATION OF SHRIMP SHELLS CHITOSAN PRODUCING CONDITIONS

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ABSTRACT: *Due to the multiapplications of chitosan particularly in food products. This paper was preformed to achieve the optimum parameters involved in shrimp shells processing. The shells had 44.96% and 36.63% ash and protein respectively. Different concentrations of HCl were used to remove ash (demineralization) which 2M HCl at 45°C for 2hr was the best with ash reduction rate of 91.98%. Removal of protein (Deproteinization) was optimum at 1M NaOH at 75°C for 4hr. To produce chitosan deacetylation of chitin is required and the optimum parameters were 40% (10M NaOH) at 90°C for 2hr.*
The produced shrimp shells chitosan under these conditions had 83.53%, 521.65% and 405.65% degree of deacetylation, water and fat binding capacities respectively.

Keywords: *Shrimp shells, chitin, chitosan, deacetylation, functional properties.*

INTRODUCTION

Approximately 70% of the landed value of shells fish 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 biochemical's and nutrients, such as chitin, pigments, seafood peptones, ….etc., from these by products (Seo *et. al.,* 2007). Chitin is found in marine invertebrates, insects, fungi …etc., 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 shells fish. Some fungi contain chitosan; however, it is commercially produced by the deacetylation of chitin (Tsugita, 1990).

Chitosan is a natural biopolymer derived by deacetylation of chitin, a major component of the shells of crustacean such as crab, shrimp, and crayfish. During the past several decades, chitosan has been received increased attention for its commercial applications in biomedical, food, and chemical industries (Sandford and Hutchings, 1987). Chitosan is now widely produced commercially from crab and shrimp shells wastes.

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 shells 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 shells 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 (Gang *et. al.,* 2010 and Jung *et. al.,* 2006).

Therefore this study aimed to evaluate define the optimum parameters (acid / alkaline concentration, temperature and time) to extract chitin and chitosan from shrimp shells. The chemical compositions, functional properties of shrimp shells chitosan were studied.

MATERIAL AND METHODS

1. Materials

1.1. Source of shrimp shells

Shells of green shrimp *Caridina babaulti* 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 it freeze immediately (at -18°C) and stored for further analysis.

1.2. Chemicals and Reagents:

Hydrochloric acid, glacial acetic acid, sodium hydroxide, phenolphthalein, methyl orange, were purchased from El-Nasr Pharmaceutical Chemicals, El-Ameriea, Cairo, Egypt. Aniline blue was purchased from ROTH Bestellen sie zum (Nulltarif, Germany), Sulphoric acid was obtained from Merck, Darmstadt, Germany.

2. Methods

2.1. Chitosan producing methods

2.1.1. Preparation of shrimp shells

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

2.1.2.Demineralization of shrimp shells

A preliminary experiment to define the optimum demineralization condition of shrimp shells was carried out using 50g dried shrimp shells. Demineralization was carried out using 1M HCl at 45 °C for 2 hr with a solution/solid ratio of 1/15 v/w. The HCl which showed the highest ash reduction rate (2M) was applied during determination of the optimum temperature (45°C).The temperature (45°C) resulted in the highest ash 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.

2.1.3.Deproteinization of shrimp shells

A preliminary experiment to define the optimum conditions for deproteinization of demineralized shrimp shells and deproteinized was carried out using 50g demineralized dried shrimp shells. Deproteinization was carried out using NaOH concentrations (1M NaOH) at a temperature of 75 °C for 4 hr 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 ash reduction rate was applied during determination of the optimum treatment time (4hr).

The optimum conditions for deprotienization treatment of chitin were 1M NaOH at 75°C for 4hr.

2.2. Preparation of chitosan

2.2.1. Deacetylation of shrimp shells chitin

A preliminary experiment to define the optimum deacetylation condition of shrimp shells chitin was carried out using 50g dried shrimp shells chitin (demineralized and deprotienized). Deacetylation was carried out using different NaOH concentrations (40% NaOH) at a temperature of 90 °C for 4hr with a solution/solid ratio of 1/15 v/w. The NaOH which showed the highest degree of deacetylation (D.D %) by using 40% NaOH, was applied during determination of the optimum temperature (90°C). The temperature (90°C) resulted in the highest % degree of deacetylation (D.D%) was applied during determination of the optimum treatment time (2hr).

Finally the optimum conditions 40% NaOH, 90°C and 4hr which recorded the highest % degree of deacetylation (D.D %) were applied to produce shrimp shells Chitosan.

2.3. Physico-chemical and functional properties methods

2.3.1. Proximate composition

The procedure of AOAC (2003) 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.

2.3.2. Measurement of Degree of Deacetylation (% DD)

The acid–base titration method was used to determine the DD from the amino group content in chitosan. Chitosan (0.3 g) was dissolved in 30 ml of HCl standard solution (0.1 mol/L). Methyl orange and aniline blue mixing indicators were added. A standard solution of 0.1M NaOH was used for titration until the solution became blue green. The following formulas were used to calculate the DD: (Luo *et. al.,* 2000).

$$(-NH_2)\% = \frac{0.016(C_1V_1 - C_2V_2)}{W} \times 100$$

$$DD\% = \frac{203(-NH_2\%)}{16 + 42(-NH_2\%)} \times 100$$

Where C1, V1, C2, and V2 are the concentrations and volumes for the HCl standard solution and NaOH standard solution, respectively, and W is the weight of the sample.

2.3.3. Solubility, Water Binding Capacity (WBC) and Fat Binding Capacity (FBC) of chitosan

2.3.3.1. Solubility

Chitosan (0.1 g) was placed into a centrifuge tube (known weight) then dissolved with 10 ml of 1% acetic acid for 30 min using an incubator shaker operating at 240 rpm and 25ºC. The solution was then immersed in a boiling water bath for 10 minutes, cooled to room temperature (25°C) and centrifuged at 10,000 rpm for 10 min. The supernatant was decanted. The residue particles were washed with distilled water (25ml) then centrifuged at 10,000 rpm. The supernatant was removed and the residue dried at 60°C for 24hr. Finally, weighed the dried residue and the percentage of solubility was calculated as followed:

$$\% \text{ Solubility of Chitosan} = \frac{\text{(Initial weight of tube + chitosan) - (Final weight of tube + chitosan)}}{\text{(Initial weight of tube + chitosan) – (Initial weight of tube)}} \times 100$$

2.3.3.2.Water and fat binding capacity

Water binding capacity (WBC) and fat binding capacity (FBC) of chitosans were measured using the method of No *et. al.,* (2000). Briefly, the procedure was carried out by weighing a centrifuge tube containing 0.5 g sample, adding 10 mL of water or corn oil, and mixing on a vortex mixer for 1 min to disperse the sample. The contents were left at ambient temperature for 30 min with shaking for 5s every 10 min and centrifuged at 3200 rpm for 25 min. The supernatant was decanted and the tube was weighed again. WBC and FBC were calculated using the following formula:

WBC(%)= [water bound (g)/sample weight (g)]× 100.
FBC (%) = [fat bound (g)/sample weight (g)] × 100.

2.4. Statistical analysis

Statistical analysis was done using analysis of variance (ANOVA), and least significant difference (LSD) were obtained to compare the means of treatments, using Costat version 6.311 (Copyright 1998 – 2005, CoHort Software). Duncan's multiple range test (Duncan, 1955) was used to compare between the treatments means.

RESULTS AND DISCUSSION

1. Proximate composition (on dry weight basis) of crude shrimp shells

Proximate composition of crude shrimp shells (moisture, protein, fat, ash, carbohydrates, and fiber content), was presented in Table (1). The protein content was 36.63%, while ash content was 44.96%. Our results showed a low content of lipids (4.85%), while shrimp shells total fiber and total carbohydrates content were 6.18 % and 7.38 %, respectively. These results are in the same trend of Waldeck *et. al.,* (2006); Xu *et. al.,* (2008) and Mini *et. al., (*2011) who stated that dry *Crangon crangon* shells consist of 10–38% proteins, 31–44%

minerals. While ours are close to that reported by Hopkins *et. al.,* (1993).

The content of % total fiber was 29.41 % in chitin compared to 6.18 % in shrimp shells waste.

2. Demineralization treatments

2.1. Effect of HCl concentration

Generally, using different HCl concentrations significantly ($p \leq 0.05$) decreased the ash content (Table, 2) compared with untreated crude shells.

On the other side, no significant ($p > 0.05$) differences were noticed in ash 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 ash reduction rate (91.98%) compared with 1M, 3M, 4M and 5M (86.76, 86.58, 76.49 and 59.67 %, respectively). Concentration of 2 M was used for further extraction treatment. These results are similar with those reported by Yen *et. al.,* (2009); Gang *et. al.,* (2010) and Jung *et. al.,* (2006).

2.2. Effect of temperature

The highest reduction rate of ash 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 ash 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 (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

Table (2): Effect of different molarities of HCl on ash content of shrimp shells (on dry weight basis).

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

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

Table (3): Effect of different temperature Degrees on ash content of shrimp shells (on dry weight basis).

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

* Means in the same column with different letters are significantly different at (p≤0.05).

2.3. Effect of time

Generally, using different extraction times significantly affect (p≤0.05) the ash content (Table, 4), compared with untreated crude shells (44.96%).

On the other side, no significant (p>0.05) differences was noticed among extraction for 2 hr, 3 hr and 4 hr (2.53%, 3.89% and 3.22%, respectively). 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 ash reduction rate was obtained by extraction for 5hr (78.71%).

The results of Tables, 2, 3 and 4 indicated that the optimum conditions for reducing ash content of shrimp shells were extraction with 2M HCl at 45°C for 2hr.

Table (4): Effect of different extraction times on ash content of shrimp shells (on dry weight basis).

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

* Means in the same column with different letters are significantly different at (p≤0.05).

3. Deproteinization treatment (chitin production)

3.1. 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%).

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 concentrated alkali solution on one hand remove protein effectively but on the other hand may result in breakage of the N-acetyl glucosamine polymer chains of chitin (Kim *et. al.,* 2010).

Extraction of protein matter in alkaline medium (deproteinization) is traditionally done by treating shells 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 a 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 (Benjakul and Wisitwuttikul, (1994), Das and Ganesh., 2010).

3.2. 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).

Mini *et. al.,* (2011) reported that the optimum deproteination was carried out at a temperature ranged from 30 °C to 65 °C. The temperature of 75°C which represents the highest protein reduction rates were used for further deproteinization treatment.

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}	-
1M	3.44^{e}	63.93
2M	3.63 ^{de}	61.75
3M	4.71 ^{cd}	56.49
4M	4.59 ^{cd}	52.12
5M	6.93 ^{b}	28.06
L.S.D	1.11	-

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

Table (6): Effect of different temperature degrees on protein content of demeneralized shrimp shells.

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

* Means in the same column with different letters are significantly different at (p≤0.05).

3.3. Effect of time

Protein content was affected (p≤0.05) by extraction time (Table, 7). No significant (p>0.05) differences in total protein content were observed between 1hr and 2hr of extraction. Also, no significant (p≤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%).

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 (Xu *et. al.,* 2008).

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 (Austin *et. al.,* 1981).

Deprotenization (<5% residual protein in shrimp shells) is possible in combination of both mesophilic temperature (30 ◦C) and long incubation time (5hr) or at thermophilic temperature (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 (Mini *et. al.,* 2011).

Xu *et. al.,* (2008), stated that when the alkali treatment for deprotenization of shrimp shells was extended over more than 4hr, 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 decreased at higher temperatures, e.g. at 65 ◦C.

The results (Tables, 5, 6 and 7) indicated that the optimum conditions for deprotenization treatment of shrimp shells were 1M NaOH at 75°C for 4hr.

4. Deacetylation treatment (chitosan production)

4.1. Effect of NaOH concentration

Increasing the concentration of NaOH up to 10M resulted in a significant (p≤0.05) increase in the degree of deacetylation (Table 8). However, the degree of deacetylation was significantly (p≤0.05) decreased by NaOH concentration increasing than 10 M.

Table (7): Effect of different time on protein content of demineralized shrimp shells.

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

* Means in the same column with different letters are significantly different at (p≤0.05).

Table (8): Effect of different concentrations of NaOH on the degree of deacetylation and functional properties of shrimp shells chitosan.

NaOH concentration (M)	% Degree of Deacetylation (% DD)	% Water Binding Capacity	%Fat Binding Capacity	%Solubility
2.5	49.87 [e]	501.20 [bc]	422.89 [c]	67.03 [a]
5	53.20 [d]	489.94 [c]	412.32 [d]	62.51 [b]
7.5	60.53 [c]	511.01 [b]	464.73 [b]	55.37 [c]
10	75.83 [a]	526.49 [a]	487.27 [a]	51.40 [d]
12.5	72.83 [b]	506.22 [b]	415.63 [d]	47.39 [e]
L.S.D	2.55	11.64	4.17	2.60

* Means in the same column with different letters are significantly different at (p≤0.05).

Water binding capacity was significantly (p≤0.05) increased with increasing of NaOH concentration up to 10M. While, it was significantly (p≤0.05) decrease when the concentration of NaOH increased up to 12.5M. The same trend was observed in fat binding capacity. Solubility was significantly (p≤0.05) affected by concentration of NaOH. Increasing the concentration of NaOH resulted in a significant (p≤0.05) decreased in solubility.

This results are in good agreement with those obtained by Johnson and Peniston, 1982 and Kurita, 2006 who indicated that chitosan could be produced from chitin by deacetylation with highly concentrated (10-12.5M) solutions of sodium hydroxide.

The WBC values of chitosan were similar to that reported by Dal Kyoung *et. al.,* (2007) ranging from 523% to 539%.

According to Cho *et. al.,* (1998), WBC and FBC of five commercial chitosan products ranged from 458% to 805% and 314% to 535%, respectively. Water binding capacity and fat binding capacity of six

commercial chitosan products observed by No *et. al.,* (2000) were in the range of 355–611% and 217–403%, respectively. The WBC (526.49%) and FBC (487.27%) of the obtained chitosans observed in this study were comparable to those of commercial chitosans reported by Cho *et. al.,* (1998) and No *et. al.,* (2003).

4.2. Effect of temperature

Effect of temperature on the degree of deacetylation and functional properties of shrimp shells chitosan were presented in Table (9). The degree of deacetylation was not significantly ($p>0.05$) affected by the temperature of extraction up to 60°C. However, more than 60ºC resulted in a significant ($P\leq0.05$) increase in the degree of deacetylation. The highest ($p\leq0.05$) water binding capacity was noticed when the chitin extracted at 90°C (528.53%) while the lowest ($p\geq0.05$) value was observed when the chitin treated at 45°C (450.60%).

The fat binding capacity had the similar trend of water binding capacity. Solubility was significantly ($p\leq0.05$) affected by temperature. Solubility was significantly ($p\leq0.05$) increased with temperature up to 60 °C. The temperature higher than 60 °C resulted in a significant ($p\leq0.05$) decrease in the solubility. Conversion of chitin to chitosan involves a treatment with concentrated NaOH at high temperature for the removal of acetyl moieties from the N-acetylglucosamine units of chitin. Thus, the contact time of chitin with e.g. 50% NaOH for deacetylation plays a crucial role for the viscosity of chitosan (Xu *et. al.,* 2008; Waldeck *et. al.,* 2006; Mini *et. al*., 2011).

Degree of deacetylation is an important parameter affecting solubility, chemical reactivity and biodegradability. Depending on the source and preparation procedure, DD may range from 30% to 90% (Martino *et. al*, 2005).Water binding (WBC) and fat binding capacities (FBC) of commercial chitosan are lower than the extracted chitosan. Water and fat binding capacities of different commercial chitosan were reported as 458–805% and 314–535%, respectively, by Cho *et. al.,* 1998. WBC and FBC of six commercial chitosan products observed by No *et. al.,* (2000) were in the range of355–611% and 217–477%, respectively. The WBC (492.67%) and FBC (383.04%) of commercial chitosan in the present study were compatible to those reported by Cho *et. al.,* 1998; No *et. al.,* 2000.Water solubility of chitosan has been found to rely on degree of deacetylation (DD) and randomly 50% deacetylated chitosan is soluble in neutral water or even under alkaline conditions. On the other hand, water-soluble chitosan with about 50% DD can be prepared from chitosan by N-acetylating with acetic anhydride. (Prashanth *et. al.,* 2002; Jayakumar *et. al.,* 2010).

Table (9): Effect of different temperatures on the degree of deacetylation and functional properties of shrimp shells chitosan

Temp (°C)	%Degree of Deacetylation (% DD)	% Water Binding Capacity	% Fat Binding Capacity	% Solubility
30 °C	74.97 ^{c}	498.43 ^{d}	414.17 ^{b}	54.31 ^{c}
45 °C	75.16 ^{c}	450.60 ^{e}	391.36 ^{d}	60.02 ^{b}
60 °C	76.67 ^{c}	502.56 ^{c}	385.52 ^{e}	64.55 ^{a}
75 °C	79.28 ^{b}	510.51 ^{b}	407.33 ^{c}	57.70 ^{b}
90 °C	82.76 ^{a}	528.53 ^{a}	512.91 ^{a}	50.41 ^{d}
L.S.D	2.55	2.40	5.07	2.70

* Means in the same column with different letters are significantly different at ($p\leq0.05$).

4.3. Effect of time

No significant ($p>0.05$) differences were detected in the DD (%) between the chitin treated for 4hr (76.83%) and that treated for 5hr (Table 10).

The highest value of WBC was obtained when the shrimp shells chitin treated for 2 hr (537.71%), while the lower water binding capacity was detected when shrimp chitin treated for 5 hr (491.27%). Fat binding capacity (%) showed the highest value when shrimp shells chitin treated for 4 hr (424.48%), however it showed low values by using 1 hr and 2 hr (311.17% and 345.89%) respectively. The effect of heating time on the degree of deacetylation and % solubility of shrimp shells chitosan showed clear decrease in both parameters due to the increase of time.

The results (Tables, 8, 9 and 10) indicated that the optimum condition for producing shrimp shells chitosan were extraction with 10M NaOH at 90°C for 2hr. Shrimp shells chitosan produced under the optimum condition had 83.53% degree of deacetylation, 521.65% water binding capacity and 405.65% fat binding capacity.

It seems that the purity for the product to be considered as chitosan was 80.5%. Accordingly, all crab chitosans were nearly pure chitosans. For the purity of crab chitosan products, the reaction time of 60 min was sufficient (Stoscheck, 1990).Water binding (WBC) and fat binding capacities (FBC) of commercial chitosan are lower than the extracted chitosan. Water and fat binding capacities of different commercial chitosan were reported as 458–805% and 314–535%, respectively, by Cho *et al*., (1998).Water Binding capacity and FBC of six commercial chitosan products observed by No *et al., (*2000) were in the range of355–611% and 217–477%, respectively. The WBC (492.67%) and FBC (383.04%) of commercial chitosan in the present study were compatible to those reported by Cho *et. al.,* (1998) and No *et. al.,* (2000).

4.4. Effect of optimum conditions for producing shrimp shells chitosan on the degree of deacetylation and some functional properties

Table (11) illustrates the effect of optimum conditions for producing shrimp shells chitosan (10 M NaOH at 90 °C for 2hr), on the degree of deacetylation and some function properties. Shrimp shells chitosan produced under the optimum condition had 83.53% degree of deacetylation, 521.65% water binding capacity and 405.65% fat binding capacity. The solubility of the produced chitosan reached to 55.65%. The values obtaining for the produced shrimp shells chitosan are too close to those stated by several investigators. Mortino *et. al.,* (2005) stated that the degree of deacetylation is an important parameter affecting solubility, chemical reactivity and biodegradability. Depending on the source and preparation procedure, DD may range from 30% to 90%.

Table (10): Effect of different times on the degree of deacetylation and functional properties of shrimp shells chitosan.

Time (hr)	% Degree of Deacetylation (% DD)	% Water Binding Capacity	% Fat Binding Capacity	% Solubility
1 hr	85.35 ^{ab}	519.75 ^{c}	311.17 ^{e}	53.79 ^{b}
2 hr	86.67 ^{a}	537.71 ^{a}	345.89 ^{d}	59.79 ^{a}
3 hr	81.94 ^{b}	517.36 ^{c}	418.46 ^{b}	48.55 ^{c}
4 hr	76.83 ^{c}	531.69 ^{b}	424.48 ^{a}	43.71 ^{d}
5 hr	78.01 ^{c}	491.27 ^{d}	383.76 ^{c}	40.99 ^{e}
L.S.D	3.67	2.40	2.08	1.34

* Means in the same column with different letters are significantly different at ($p\leq0.05$).

Table (11): Effect of optimum conditions for producing shrimp shells chitosan on the degree of deacetylation and functional properties.

Samples	% Degree of Deacetylation (% DD)	% Water Binding Capacity	% Fat Binding Capacity	% Solubility
Chitosan	83.53	521.65	405.65	55.65

Among many characteristics, the degree of deacetylation is one of the most important chemical characteristics, which influences the performance of chitosan in many of its applications (Muzzarelli *et. al.,* 1994; Li *et. al.,* 1992 and Baxter *et. al.,* 1992). In addition, DD, which reveals the content of free amino groups in the polysaccharide (Li *et. al.,* 1992), can be used to differentiate between chitin and chitosan. Emi-Reynolds *et. al.,* (2007) reported DD value of 89.7%.

Fat binding capacity signifies how the chitosan can easily bind or absorb fat especially when used in the manufacturing of dietary supplements. The trend recorded for water binding capacity was similarly observed for fat binding capacity. Values for un-irradiated and irradiated shrimp chitosan were 560.55% and 431%, respectively, for local frytol, while that for the commercial chitosan samples (un-irradiated and irradiated) were 490.10% and 529.05%, respectively. Rout (2001) reported that the average fat binding capacities of craw fish chitosan and commercial crab chitosan for soybean oil were 706% and 587%, respectively. The values obtained in this research were lower than the values reported by Rout (2001).

Water binding (WBC) and fat binding capacities (FBC) of commercial chitosan are lower than the extracted chitosan. Water and fat binding capacities of different commercial chitosan were reported as 458–805% and 314–535%, respectively, by Cho *et. al.,* (1998).WBC and FBC of six commercial chitosan products observed by No *et. al.,* (2000) were in the range of355–611% and 217–477%, respectively. The WBC (492.67%) and FBC (383.04%) of commercial chitosan in the present study were compatible to those reported by Cho *et. al.,* (1998) and No *et. al.,* (2000).

Chitin was subjected for deacetylation using alkali where the acetamide group was converted into amino group and nitrogen content (%) showed obvious increase which further increased when the deacetylation process was repeated. The degree of deacetylation of the chitosan after second deacetylation was higher than that of chitosan after first deacetylation as reflected in the nitrogen content values. (Teli and Javed, 2012).

4.5. Effect of optimum conditions for producing shrimp shells chitosan on its chemical composition

Estimation of the proximate composition of the produced shrimp shells chitosan is represented in Table (12).

Table (12): Proximate composition (on dry weight) of crud shrimp shells and shrimp shells chitosan:

Samples.	%Moisture	%Protein	% Fat	%Carbohydrates	% Ash	%Fiber
Washed crud shells	13.05	36.6	4.85	7.38	44.96	6.18
Chitosan	8.81	1.23	0.54	64.18	0.26	33.60

Generally, the shrimp shells chitosan had a lower content of total ash, total protein and total fat (0.26%, 1.23% and 0.54%, respectively) compared with the crude shrimp shells (44.96%, 36.60% and 4.85%, respectively). While, the chitosan showed higher total fiber content (33.60%) compared with crude shells (6.18%). Amount of protein content was present in cuttlebone (30 - 32%). At the same time no protein content was found in chitosan as indicated by no absorbance at 280 nm (Aruldhason *et. al.,* 2012).

Conclusion:-

The best condition for shrimp shells demineralization process was 2M HCL at 45 ºC for 2hr. Meanwhile, the best condition to remove the protein from shrimp shells was 1M NaOH at 75 ° C for 4hr.meanwhile, the best condition to production of shrimp shell chitosan was 10M NaOH at 90 ° C for 2hr.

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"الظروف المثلى لإنتاج الكيتوزان من قشور الجمبرى"

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الملخص العربى:

نظرا للإستخدامات المتعددة والتطبيقات الحديثة فى إستخدام الكيتوزان فى العديد من المنتجات الغذائية هدفت هذه الدراسة الى التعرف على الظروف المثلى لإنتاج الكيتوزان من قشور الجمبرى ، حيث أظهرت النتائج أن قشور الجمبرى تحتوى على 44,96% رماد و36,63% بروتين ، وعند إستخدام تركيزات مختلفة من حامض الهيدروكلوريك للتخلص من الرماد (إزالة الرماد) أظهرت النتائج أن تركيز 2مولر من حامض الهيدروكلوريك أعطى أفضل النتائج فى التخلص من الرماد وذلك على درجة حرارة 45°م لمدة 2 ساعة حيث بلغت نسبة الإنخفاض فى الرماد الى 91,98% مقارنة بالقشور الخام.

كما أظهرت النتائج أيضا أن إستخدام تركيز 1 مولر من محلول هيدروكسيد الصوديوم على درجة حرارة 75°م لمدة 4 ساعات أعطى أفضل النتائج فى إزالة البروتين. ووجد أن أستخدام تركيز 10 مولر من محلول هيدروكسيد الصوديوم على درجة حرارة مقدارها 90°م لمدة 2 ساعة أعطى أفضل النتائج فى عملية تحويل الكيتين الى الكيتوزان (إزالة مجموعة الأسيتيل) ، ووجد أن الكيتوزان الناتج تحت هذة الظروف درجة تحويلة من الكيتين الى الكيتوزان بلغت حوالى 83,53% ووجد أن درجة إرتباط الكيتوزان الناتج مع الماء والدهن بلغت حوالى 521,56% ، 405,065% على الترتيب.