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Production and Evaluation of Some Bioactive Compounds Extracted from Squilla (*Oratosquilla massavensis*) Shells

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Abstract: This work was performed to investigate the production and physicochemical properties of some bioactive compounds (chitin, chitosan and astaxanthin) extracted from squilla (*Oratosquilla massavensis*) shells. Chemical composition of squilla shells and chitin yield were determined. Results showed that pre-treated squilla shells contained 68.11% moisture, 12.88% true protein, 4.79% crude fat and 44.59% ash content (on dry weight). Chitin yield and its ash content ranged between 15.75–16.08% and 0.81–1.26%, respectively. The physicochemical properties of chitosan at different times and temperatures showed that chitosan composed 8.73–11.19% moisture, 0.66–0.83% true protein, 0.14 - 0.25% ash content. Viscosity of chitosan at higher temperatures (120°C and 130°C) for different times (30 min and 60 min) were significantly ($P<0.05$) lower (80-111cps) than the lower temperatures (100°C and 110°C) for 60 min (138-130cps). At 120°C and 130°C, for 30 min, solubility was significantly lower (93.5-95.6%) than all other temperatures and times used. The degrees of deacetylation (DD) were significantly different at 130°C for 30 min and 60 min than all other temperatures and times used being higher than 70 % and ranged 73.11%-84.68%. Average molecular weight (MW) of chitosan at 120°C for 30 min was significantly different than all times and temperatures used except 120°C for 60 min and as high as 130°C for 60 min. Thus, it is obvious that MW ranged 180-189 Kilo Dalton was significantly different than lower Mw value (134.8 KD). A high value of water binding capacity (WBC) was found at 120°C for 60 min while fat binding capacity was found at 120°C for 30 min and 60 min compared with other treatments. Concerning the carotenoids, it was found that the astaxanthin in female gonads exhibited higher carotenoid concentration (14.01µg\g) than the shells (10.10µg\g on wet weight). In conclusion, squilla shells are highly prized as an inexpensive market value which could be converted into a valuable expensive chitosan and female gonads are considered a good source for carotenoids, particularly astaxanthin.

Keywords: Physicochemical, Properties, Squilla, Chitin, Chitosan, Astaxanthin

1. Introduction

Huge quantities of inexpensive varieties of squilla *spp (Crustacea, squillidae)* are caught as by-catch and they are not properly utilized. The total squilla *spp.* production from only the Mediterranean Sea catch during 2013 was 215 tons [1]. Chitin extracted from the exoskeleton of crab, squilla, and shrimp, could be used in a variety of applications especially when transformed into the more useful compound chitosan. The increased use of the biopolymer, have been gaining importance as raw materials in many industrial sectors such as food, textile, packaging, medicine and pharmacy. Biopolymers play crucial roles in applications where the materials are in direct contact with body tissue [2]. Also, chitin (*β*-(1-4)-2-acetamido-2-deoxy-D-glucopyrnaose) is the second most abundant natural biopolymer generally obtained from exoskeletons of crustaceans such as crabs, shrimp, lobsters and krill. The most important derivative of chitin is chitosan ((1, 4)-2-amino-2-deoxy-*β*–D glucose) obtained by excessive deacetylation of chitin with alkali. Chitosan, the linear polymer of D- glucosamine in *β*-(1-4) linkage has been recommended as a suitable functional material because of its biocompatibility, biodegradability, non-toxicity, adsorption properties and regulation of cell activation. Due to its antimicrobial activity, chitosans have

many pharmaceutical applications and generally used as natural preservative for the safety of food [3-5]. Crustacean exoskeleton is an important natural source of carotenoids, particularly astaxanthin. Several studies have been carried out to recover the pigment from crustacean processing discards. Methods of extraction of carotenoids using organic solvents and edible oils have been attempted [6].The antioxidant activity of carotenoid-containing protein isolate from crustacean wastes was studied. The conditions for isolation of antioxidant-rich carotenoprotein from shrimp heads using an autolytic process were optimized. Also, the antioxidant activity of shrimp carotenoid extract indicated its potential for use as a natural antioxidant for use in food and biomedical applications [7]. In the family of crustaceans, squilla is another candidate that has not been explored as a source of chitin and chitosan. It is a by-catch with an extremely low economic value and is available in large quantities in the seas of countries in the tropical regions [8]. Therefore, the current research was performed to make the utmost utilization of *Oratosquilla massavensis* for the production of some bioactive component. This can be achieved in the present study by extracting chitosan from the shells and astaxanthin from both shells and gonads and determine the physicochemical properties of the extracted chitosan.

2. Materials and Methods

About 30 kg fresh *squilla* (*Oratosquilla massavensis*) samples were purchased from the commercial catch at El-Anfoshy landing place, Alexandria, Egypt during the period from May to December, 2014. They were transported using icebox to the Fish Processing and Technology Lab., National Institute of Oceanography and Fisheries, Alexandaria. Fresh squilla samples were frozen at -30°C in an Air Blast Freezer for about 10 minutes, after thawing the shells were removed by scissors from the abdominal region up to the thoracic region. Flesh was utilized to obtain some fishery products. Shell wastes was packed in polyethylene bags and stored at -18°C until utilized. Frozen shells were thawed at ambient temperature, steamed for 10 min, blended with tap water (1:1w\v) using a mixer (Malounix, Jeannette 243 France) to remove undesirable organic matters, adherent proteins and other impurities. Samples were dried at 70°C overnight then grinded. Ground shells were placed in tightly closed glass jars and stored at ambient temperature until analysis.

2.1. Extraction of Chitin and Chitosan

Two techniques were performed for chitin extraction as reported by [9]. Squilla shells were subjected to two production techniques (A and B). In technique A; demineralization by 3% HCl was done twice for 3 h and 4% NaOH treatment during 24 h was given between the two HCl treatments. As inverse to technique A, the technique B was started with deproteinization with 4% NaOH twice (12h for each period) HCl treatment (4.5%, 6 h) was given in between. In the present study, chitosan was obtained from chitin extracted from technique A by deacetylation (50% NaOH at 100,110°C for 60 min and 120,130 °C for 30-60 min. Fig. (1) shows the technique steps of chitosan production as described by [10].

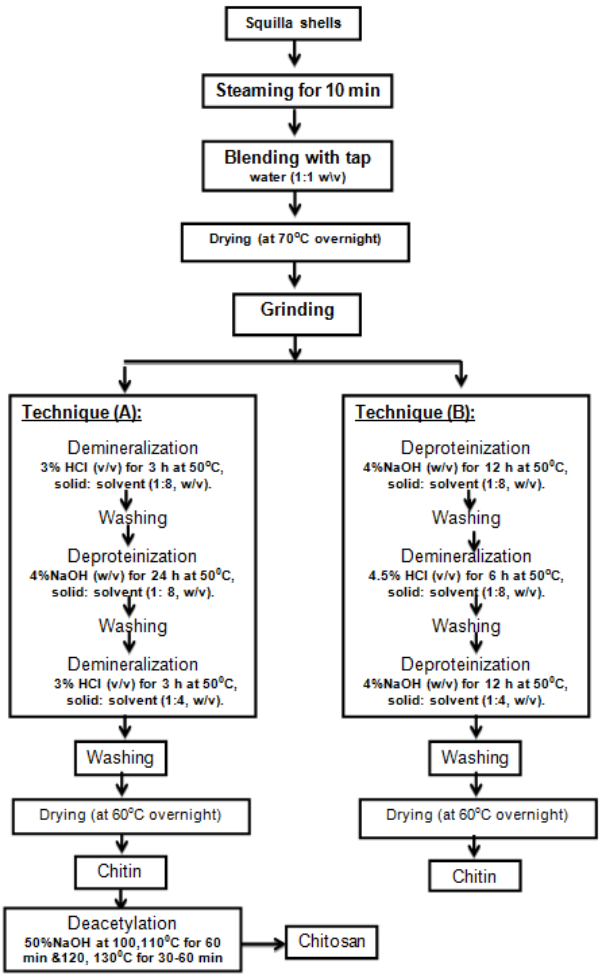

Fig. (1). *Flowchart of chitosan extracted from squilla shells.*

2.2. Extraction of Carotenoids (as Astaxanthin)

Total carotenoids were determined according to the method described by [6]. A known weight of homogenized wet squilla shell (2g) was extracted with 20 ml of acetone and centrifuged at 3500 rpm for 5 minutes. The supernatant was conserved while the precipitate was re-extracted several times, until the acetone extract became colorless. The pooled supernatant was transferred to approximately 25 ml of petroleum ether in a separation funnel. The petroleum phase, containing the carotenoids, was washed several times with 2% (w/v) NaCl solution, filtered through anhydrous sodium sulphate and diluted to 25 ml final volume with petroleum ether. The absorbance (A) of the carotenoid extract in petroleum ether was measured at 467 nm. Total carotenoid (as astaxanthin of wet squilla shell) was calculated as:

Total carotenoid (as astaxanthin) $\mu g g^{-1} = (A_{467})\ (D)\ (V)\ /0.2400\ (W)$

Where: A_{467} = The absorbance at 467 nm in a cm light path; D = Dilution factor of sample prior to spectral analysis; V = The volume of petroleum ether containing pigments (normally 25 ml); W = Weight of sample; and Value (0.2400) = The extinction coefficient of astaxanthin in petroleum ether $E_{1\%\ 1cm}$).

2.3. Analytical Methods

2.3.1. Chemical Composition of Squilla Shells

Chemical composition as moisture content, crude fat and ash content of the shells were determined according to [11]. True protein was determined by using standard Biuret protein assay with Bovine Serum Albumin (Sigma, St. Louis, MO) as standard [12]. Moisture content of the extracted chitosan was determined at 60°C overnight according to the gravimetric method as described by [13].

2.3.2. Degree of Deacetylation (DD

Degree of Deacetylation (DD) of chitosan was characterized by Fourier Transform Infrared (FTIR) Spectroscopy in the range of 500 to 4000 cm-1(Brucker Tensor 37) as described by [14]. Ten µg samples were mixed with 100 µg of dried Potassium Bromide (KBr) and compressed to prepare a salt disc (10 mm diameter). The absorbance of IR spectrum at 1637 and 3430 cm^{-1} were measured to calculate the DA according to the following equation:

$$DA\ (\%) = 100 - (A_{1637}/ A_{3430}) \times 115$$

$$DD\ (\%) = 100 - DA\ (\%)$$

2.3.3. Average Molecular Weight

Molecular Weight (MW) average of the viscosity (Dalton) was determined, the chitosan was dissolved in a mixture of 0.1 M acetic acid with 0.2 M NaCl, and then the automated solution viscometer was used to measure the intrinsic viscosity (η). The Mark-Houwink equation relating to intrinsic viscosity with empirical viscometric constants $K=1.81X10^{-3}$ cm^3/g and a = 0.93[15] for chitosan was used to calculate the molecular weight using the following equation: $[\eta] = KM^a$

2.3.4. Viscosity of Chitosan

Viscosity of chitosan was determined with a Myr Rotary Viscometer Series VR 3000 (Model V1-L) as reported by [16]. Chitosan solution was prepared in1% acetic acid at a 1% concentration on a dry basis. Measurement was made in duplicate using a No. 1 spindle at 50,100 rpm on solutions at 25°C with values reported in centipoises (cps) units.

2.3.5. Solubility of Chitosan

Solubility of chitosan was measured using the modified method of [17] and determined by dissolving 1% (w/v) chitosan in a solution of 1% glacial acetic acid with continuous stirring for 24 h. The solution was centrifuged to determine the % insoluble chitosan. Squilla chitosan powder (0.1 g in triplicate) were 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 25°C. 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 undissolved particles were washed in distilled water (25 ml), and then centrifuged a 10,000 rpm. The supernatant was removed and undissolved pellets dried at 60°C for 24 hr. Finally, the particles were weighed and determined the percentage solubility according to the following equation:

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

2.3.6. Water and Fat Binding Capacities (WBC & FBC)

Water and fat binding capacities (WBC & FBC) of chitosan were measured using a modified method of [18]. WBC & FBC were initially carried out by weighing a centrifuge tube containing 0.5 g of sample, adding 10 ml of water, and mixing on a vortex mixer for 1 min to disperse the sample. The contents were left at ambient temperature for 30 min with intermittent shaking for 5 sec every 10 min and 38 centrifuged at 3,500 rpm for 25 min. After the supernatant was decanted, the tube was weighed again. WBC & FBC were calculated as follows:

WBC (%) = [water bound (g)/ initial sample weight (g)] x 100.

FBC (%) = [fat bound (g)/ initial sample weight (g)] x 100.

2.4. Statistical Analysis

The obtained data were used for descriptive statistical analysis consisting of means± standard deviation of triplicates. In order to test the significance of physic -chemical properties of chitosan, one-way ANOVA test were applied and means were further differentiated by Duncan's Multiple Range Test [19] (SAS,2001). Means with the same letter for each parameter are not significantly different, otherwise they do ($P<0.05$).

3. Results and Discussion

3.1. Chemical Compositions of Squilla Shells

The chemical compositions of squilla (*O. massavensis*) shells are presented in Table (1).

Table (1). *Chemical composition of squilla shells.*

Constituent (%)	Wet weight (WW)	Dry weight (DW)
Moisture content	68.11±0.69	---
True protein*	4.11± 0.41	12.88±0.31
Crude fat	1.53±0.19	4.79±0.59
Ash content	14.22±0.14	44.59±0.44

Data are expressed as mean ± S.D. of triplicates
* Determined by the method of Biuret [12].

Squilla shells contained 68.11% moisture, 12.88% protein, 4.79% fat and 44.59% ash (dry weight). Also, results in the present study are in accordance with the findings of [16] who

reported that the chemical composition of squilla (*S. empusa*) shells were 71.3% moisture, 21.2% protein and 42.8% ash. In addition, [20] reported that the proximate composition (dry basis) of prawn waste was 75-80 % moisture, 30-35 % ash, 35-40 % protein and 3-5 % fat.

3.2. Chitin Yield and Ash Content

Chitin yield and ash content of chitin. Table (2) shows the effect of two extraction techniques on chitin, chitosan yield and ash content.

Table (2). *Effect of extraction techniques on Chitin yield and ash content of chitin.*

Constituent (%)	Technique (A)	Technique (B)
Chitin yield	15.75±0.21	16.08±0.09
Ash content	0.81±0.11	1.26±0.23
Chitosan yield	86.50	87.00

Data are expressed as mean ± S.D. of triplicates
Technique (A): decalcification by 3% HCl was done twice for 3 h each and 4% NaOH treatment during 24 h was given between the two HCl treatments.
Technique (B): the squilla shell was deproteinated with 4% NaOH twice for 12-h period each and HCl treatment (4.5%, 6 h) was given in between.

Results show that squilla shells from the pilot study represent about 60% by weight of the whole squilla whereas, the edible portion comprise 40%. Results also show that Chitin yield in the present study ranged between 15.75 - 16.08 % and its ash content ranged between 0.81 - 1.26%, in addition, chitosan yield ranged 86.5 – 87.00% according to techniques (A&B**)** applied, respectively (Table2).

These results are in agreement with [21] who reported that the average head and shell waste yield from the shrimp is around 60 % by weight of the whole shrimp. And, it contained 15 - 40% chitin. Whereas, [22] reported 12 -16% chitin content from squilla shell. As the matter of fact, [20] reported 15-20 % chitin content from prawn waste. [23] showed that the chitin content of the different sources may vary over a large range (from 7% with barnacles to 40% with squid pens). On the other hand, in this study, the yield of chitin extracted from squilla shells by two techniques was higher than those reported by [24] who found that the yield of chitin obtained from 20 g *Oratosquilla nepa* shell was 2.145g (10.725%) while the shell yield of *Oratosquilla quinquedentata* was 2.125g (10.625%).

3.3. Physicochemical Properties of Chitosan

The effect of both temperature and time using the conditions adopted in the flowchart of chitosan Fig (1), deacetylation by 50% NaOH on physicochemical characteristics of chitosan are shown in Table (3).

Table (3). *Effect of both temperature and time on physicochemical characteristics of chitosan.*

Treatments (temp.& time)	Moisture (%)	Ash (%)	Protein (%)	Viscosity (cps)	Solubility (%)	*DD (%)	**Mw (KD)
100°C (60 min)	8.73±0.48^{a}	0.135±0.03	0.758±0.13	138±2.83^{a}	98.27±0.67^{ab}	75.79±0.26^{d}	189.290±42.78^{a}
110°C (60 min)	11.19±0.12^{b}	0.254±0.05	0.825±0.13	130±4.24^{a}	97.25±0.35^{ab}	73.11±0.84^{e}	184.174±24.43^{a}
120°C (30 min)	10.85±0.60^{b}	0.218±0.04	0.824±0.13	80±2.83^{e}	93.5±2.12^{c}	82.25±0.11^{b}	134.855±27.61^{b}
120°C (60 min)	10.91±0.02^{b}	0.201±0.03	0.724±0.16	100±4.24^{d}	98.8±0.57^{a}	84.68±0.82^{a}	172.335±39.36^{ab}
130°C (30 min)	11.04±0.04^{b}	0.249±0.02	0.656±0.13	120±3.54^{b}	95.6±0.85^{bc}	77.82±0.86^{c}	180.843±17.49^{a}
130°C (60 min)	10.67±0.13^{b}	0.196±0.09	0.662±0.13	111±1.41^{c}	97.67±1.31^{ab}	78.65±0.64^{c}	163.943±29.38^{ab}

Mean with different superscripts in a row or columns are significantly different at ($p< 0.05$)
*DD: degree of deacetylation
Deacetylation process in all treatments (time / temp) were performed using 50% NaOH
**Mw: average molecular weight (kilo Daltons)

3.3.1. The Chemical Compsation of Chitosan

The values of the moisture content of chitosan were 8.73, 11.19, 10.91, and 10.67% at 100, 110, 120°C and 130°C for 60 min, respectively, whereas, values were 10.85 and 11.04% at 120°C and 130°C for 30min, respectively. On the other hand, results indicated that the moisture content at low temperature (100°C for 60 min) was significantly lower than all other temperatures and times. Chitosan is hygroscopic in nature [25], hence it is very possible that the chitosan samples in the present study may be affected by moisture absorption during storage. According to [26], commercial chitosan products contain ≤ 10% moisture content. Concerning the ash content, values were 0.135, 0.254, 0.201 and 0.196% at 100, 110, 120°C and 130°C for 60 min, respectively. Also, values were 0.218 and 0.249% at 120°C and 130°C for 30 min, respectively. The ash content in chitosan is an important parameter. Some residual ash of chitosan may affect their solubility, consequently contributing to lower viscosity, or can affect other more important characteristics of the final product. A high quality grade of chitosan should have less than 1% of ash content and less than 1% protein [10]. With regard to true protein content, the values were 0.758, 0.825, 0.724 and 0.662% at 100, 110, 120 and 130oC for 60 min, respectively. Besides, for 30 min, the values of true protein were 0.824 and 0.656% at 120 and 130oC, respectively (Table 3). These results are in agreement with those reported by [16] who found that the chemical composition of chitosan extracted from squilla shells ranged between 10.4-10.7% moisture, 0.88-0.94% protein, and 0.14-0.23% ash content.

3.3.2. Viscosity

Viscosity of chitosan extracted from squilla shells, in the present study were 138, 130, 100 and 111 cps at 100, 110, 120°C and 130°C for 60 min, respectively. Also, values were 80 and 120 cps at 120 and 130°C, for 30 min, respectively (Table 3). Higher temperatures (120°C and 130°C) with

different times (30 min and 60 min) were significantly different from the lower temperature (100°C and 110°C) for 60 min. [27] reported that the viscosity of chitosan solutions in the literature generally ranges between 60 -780 cps. In the present study under the different time and temperature conditions used viscosity ranged between 80 -138 cps. [28] reported that the chitosan viscosity was closely related to the deacetylation time and that the chitosan reached its highest viscosity with a short deacetylation time.

3.3.3. Solubility

Solubility Chitin is hydrophobic (water insoluble) as well as in most organic solvents. In contrast chitosan is soluble in dilute organic acids at low pH due to the free protonable amino groups present in the D-glucosamine units [29]. The percent of solubility of chitosan in the present study were 98.27, 97.25, 98.8 and 97.67% at 100, 110, 120°C and 130°C for 60 min, respectively. Whereas, they were 93.5 and 95.6% at 120°C and 130°C, for 30 min respectively (Table 3). These results are in agreement with [16] they reported that high solubility chitosan ranged between 97.3%-98.6% obtained from *Squilla empusa*. [30] reported that the main limitations in the use of chitosan in several applications are its high viscosity and low solubility at neutral pH. Results in the present study indicated that at 120°C and at 130°C for 30 min solubility was significantly lower than all other temperature & times used, this may indicate that at lower time (30 min) low solubility may be achieved. As a matter of fact high solubility value is reached at 120°C for 60 min being 98.85%.

3.3.4. Deacetylation Degree (DD)

The deacetylation degree (DD) of chitosan is important for its use in the industry [26, 31]. Different temperatures and times used in the present study in determining the degree of deacetylation were significantly different than 130°C for 30 min and 60 min. In the present study the DD were higher than 70 % ranging between 73.11%-84.68%. According to [10], DD of chitosan ranges between 56% - 99% with an average of 80%. From this regard, certain researchers suggested that the term chitosan should be used when the degree of deacetylation is above < 75% [32]. [33] found that the DD of the commercial chitosan and the extracted chitosan from *M. stebbingi* shells were determined as 86.92% and 92.19%, respectively, by the elemental analysis and potentiometric titration.

3.3.5. Chitosan Average Molecular Weight (MW)

The molecular weight (MW) of chitosan is one of the most important properties as it considerably affects the physicochemical and functional properties [34]. In the present study, temperature at 120°C for 30min was significantly different than all times and temperatures used except 120°C for 60min and as high as 130°C for 60 min. Thus, it is obvious that Mw ranging from 180-189 KD is significantly different than lower Mw values. [33] showed that the difference in the molecular weight is caused by the difference in the deacetylation degree, and the different sources of the chitosan. In addition, several factors in the production of chitosan, such as the high temperature, concentration of alkali, reaction time, previous treatment of the chitin, particle size, chitin concentration, dissolved oxygen concentration and shear stress may also influence the molecular weight of chitosan [26,35]. It was also reported by [26] that the molecular weight of commercial chitosan ranged between 100,000 to 1,200,000 Daltons. In a study, [36] evaluated the antibacterial properties of chitosan with different MW (55 to 155 KD) but with same degree of deacetylation (80% ± 0.29), against *E. coli* with different concentrations. According to their result, all chitosans had antibacterial activity at concentrations over 200 ppm though the antibacterial activity of low MW chitosan was higher than that of the high MW samples. [37] measured the antimicrobial activity of chitosan and chitooligosaccharides with different MW without acetylated groups. They observed high MW chitosans showed strong antimicrobial activity against Gram-positive bacteria, whereas, chitosans of 11 KD and 20-30 KD molecular weights were most effective against Gram-negative bacteria. [38] reported that the effect of chitosan with MW below300 KD on Staphylococcus aureus was strengthened as the MW increased whereas, the antimicrobial effect on E. coli increased as the MW was decreased.

3.3.6. Water Binding Capacity (WBC) and Fat Binding Capacity (FBC)

The effect of extraction conditions on water binding capacity (WBC) and fat binding capacity (FBC) of chitosan is presented in Table (4). Different temperatures and times were attempted to obtain the best conditions for WBC and FBC of chitosan. The results showed that at 100°C for 60 min both (WBC) and (FBC) were significantly lower than all other time and temperature conditions. Results also indicated that by increasing temperature starting from 110°C upwards at different times (30 min and 60 min) both (WBC) and (FBC) increased insignificantly. In the present study, the WBC and FBC under different conditions of temperature and time (of chitosan extracted from sqiulla shells) were higher (672.97 - 863.24% and 503.01 - 661.79%, respectively) than those findings by [39,33] who reported that the water and fat binding capacities of different commercial chitosan were 355-805% and 217-535%, respectively. Therefore, high percent of WBC and FBC confirms the fact that chitosan extracted from squilla shells is of a good quality.

Table (4). *Effect of time and temperature on water binding capacity (WBC) and fat binding capacity (FBC) of Chitosan extracted from squilla shells.*

Treatments	WBC (%)	FBC (%)
100°C (60 min)	672.98±29.32^{c}	503.05±6.58^{c}
110°C (60 min)	863.24±9.77^{a}	613.65±19.30^{ab}
120°C (30 min)	832.78±15.51^{a}	661.79±84.00^{a}
120°C (60 min)	802.68±38.91^{ab}	527.22±9.74^{bc}
130°C (30 min)	742.55±44.78^{bc}	564.75±19.87^{bc}
130°C (60 min)	776.12±51.17^{ab}	522.60±17.82^{bc}

Mean with different superscripts in a row or columns are significantly different at ($p< 0.05$)

3.4. Caroteinoed (as Astaxanthin)

The astaxanthin content obtained from Squilla shells and female gonads is presented in Table (5). Results showed that squilla gonads had more content of astaxanthin (14.01(µg /g wet wt.) than shells (10.10 µg /g wet wt.). Therefore, gonads of squilla are a better source of astaxanthin compared to their shells which may be a promising source of astaxanthin. It was reported by [40] [41] that astaxanthin is the main ketocarotenoid responsible for the red–orange color in salmonids and crustacean and they found that astaxanthin was associated with reproduction and embryo development and also with protecting cells against oxidative damage. [40] reported that waste of *P. borealis* shrimp produced a yield of 14.8 mg/100 g dry weight. The different species of deep sea shrimps Aristeus alcocki shell waste is an excellent source of astaxanthin [42].

Table (5). *Astaxanthin content obtained from Squilla shells and gonads.*

Source	Astaxanthin (µg /g wet wt.)
Shells	10.1±0.33
Gonads	14.01±1.30

Data are expressed as mean ± S.D. of triplicates

4. Conclusion

Squilla is an inexpensive and useful raw material for flesh and the production of chitin, chitosan and astaxanthin. Chitosan produced in the present study exhibit a high degree of deacetylation, a high solubility and an attractive molecular weight, and viscosity. Thus, squilla shells are promising raw material for the extraction of bioactive compounds (chitin and chitosan) as well as considerable amounts of natural carotenoids, mainly astaxanthin from the female gonads. It has been shown also from this study, that chitosan present a great variety of properties, allowing them to have a large number of applications, but at the same time the very complex behavior of these polymers is difficult to control. This study will attract the attention of entrepreneurs, industrialists, academicians and environmentalists.

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