Evaluation of protein in persian Gulf Blue crab (*portunus pelagicus*) and The Effect of some Biological parameters on it

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Abstract: Today role and importannce of correct nutrition Is proved in providing health and preventing some diseases. In medical field also, new researches propose limiting consumption of chemical medicines for treating side effects. Therefore biological and medical spetialists consider aquatic's meat and their processed products , because they are proved to have useful compositcs such as vitamines, mineral salts , proteins , antioxidant, and unsatuatod fat acids. In this study , nutritional value of persian Gulf blue crab is investigated considering the amount of total protein . besides extracting proteins in muscle tissue of this variety with column chromatography ,the effect of some biological parameteis are investigated on the amount of these proteins. Results showed that this species have considerable amounts of animal proteins. Also it is observed that with increasing the crab's weight , the amount of muscle tissue and protein increased. Also sex had some effect on protein amount. In such a way that always in male crabs, the amount and number of extracted fractions was higher.

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1. Introduction

Aquatic's meat such as beef and pouttry have total chemical composites such as protein, lipid, water, minerals and vitamin. Which their percentage and their components are different and some how has effect on prothein quality and their nutritional value aquatic's meat are prefrablly consumed because of following. Characteristics considering nutritional value: high digestivity, mineral salts richness such as iron, selnium, Iod, calcium, magnesium , aquatic meat's richness of $Omega_3$ fat acids such as Ecoza penthatonic (EPA) and dekoza Hexanoec acid (DHA) Which have noticeable effects on health and preventing from diseases. New researches showed that among aquatics, crabs and shrimps have special properties because of selnium. In such a way that consuming these aquatics in daily nutrition diet have noticeable effects on preventing early aging , cancer and cardio-Vascular diseases because of their richness in antioxidant compositions.For example consuming 120 gr of fresh shrimp can supply 80% of daily need of individuals. Inspite that in Iran blue swimmer crab is considered only as an incidental hunting and is not consumed only as an incidental hunting and is not consumed exept in some southern areas of the country, but in global market, Fresh hunting of this species is sold 10 dollars per a kilogram and alive is sold 20 dollars. In this research by studying this species and determing its nutritional value considering protein amount. economic justification is performed for planned hunting of this type and processing and exporting the products to other countries.

2. Material and methods

Sampling was perfomed monthiy during a year from October 2007 to the end of September 2008 in persian Gulf coasts. Sampling zone includes eastern coasts (bahrekan hunting zone) whith following specifications:

START LAT 2957/639 ENDLAT 4928/065
START LAT 9540/712 ENDLAT 4928/059

And western coasts (Bouseif and lifea hunting zones) with following specifications:

START LAT 2955/184 END LAT 4906/855
START LAT 2958/199 END LAT 4903/867

Sampling was performed with several methods such as research ship and local fishers (fishing boats) with trawler net and sampler, samples were kept in ice and transfered to labratory at maximum 24 hours. For removing mud, algaes and barnacles sticked to external skeleton , crabs were washed and then they were dryed with drying paper. after sex seperating of male and female crabs. They were divided based on wet weight in 3 groups of less than 50gr, 50-100gr, and more than 100 gr. from this stage, tests were performed separately for each of six groups.

At first beside cutting carapace and removing gills, muscle tissue was isolated and was washed with cold Tris buffer (0.05 M, pH =7.5) and 200 ml of that buffer was added to it and was homogenized with refrigrator homogenizor device for 3 minutes.

Resultant tissue mixture was centri fuged with refrigrator centrifuge for 60 minutes in 6000 g and supernatant was separated. Then again buffer Tris was added to residual layer and is mixed and was centrifuged as previous stage. After second centrifuge supernatant layer was mixed with previous stage 's supernatant and was filtered. In fact this solution was raw tissue extract which was used for extracting protein fractions.

For depositing the protein with amonium sulfate , at first 29.1 g of solid amonium sulfate was added gradually to the extract per 800 mm of extract . then mentioned extract was centrifuged with refrigrator centrifuge for 60 minutes in 4^0c and 6000 g . Residual of this stage was kept (p50). Supernatant was transferred to glass and 12.5gr solid omanium sulfate was added gradually per every 100 mm of that (getting to 70 percent of saturation) and was centrifuged for 60 minutes in 4^0 c and 6000 g. Residual of this stage (p70) was separated and was mixed with the residual of previous stage (p50) (p50+p70) . buffer Tris with amuunt of 3 time more than volume was added to resultant residual (0.05M , pH=7.5) and was mixed completely. This solutiom was dialized for desalting before performing chromatography. In such a way that above solution (buffer+p50+p70) was poured in the dialize bag and the bag containg solution was settled in a big glass containing buffer Tris (0.05 M , pH=7.5) At the whole time (12hours)buffer was mixed with a magnetic mixer and was replaced with fresh Tris. at the end, the whole surface of dialize bag was covered with sucroze and was refrigrated for dehydration for 10 hours . concentrated solution was used in next stage for chromatography as the main sample. for sepatating protein fractions in two successive stages, column chromatography pharmacia 60×2.5 with fixed phase of sephadex G100 and mobile phase of Tris buffer(chloride sedium %1 M, PH=7.5, molarite %5 and flow speed, of 40 ml/h was used.in the second stage of chromatography, pharmacia column with following specifications was used: 60×1.5, fixed phase of cellulose gel DEAE and mobile phase of salty Tris buffer and 40 ml/h speed . for measuring total protein of each fraction, two methods were used: bioret and spectometric method At first sample were prepared according to following table in bioret method .

Above samples were kept in labratory environment for 5 minutes .and then device adsorption amount became zero with the evidente and absorption amount of standard pipe (Bovin Saline albomin 20 percent)was read and registered. Then optical absorption amount of all samples was read in wave length of 540 nanometer .The amount of total protein in each fraction was calculated and registered based on formula.

Tabel 1. Preparing pipes and compositions for bioret test

Preared solution	evidence	standard	Type of compositions
1000µ	1000µ	1000µ	indexkit
-	-	20µ	standard
20µ	-	-	sample
-	20µ	-	Diluted water

In spectometry method, amount of absorption in each sample was read in wavelenght of 280 nanometer and 260 nanometer .Then protein concentration of each unknown sample was calculated and registered with following formula:(Axford method 2008).

Concentration of sample protein =
[1.44×(absorption of 280)]-[0.76×(absorption in 260)]

3. Results

Total protein amounts was measured for raw tissue extract and 4 groups of fractions resultant from the first column . Chromatography results of

the first column (gel filteration) showed the existence of 4groups of protein fractions in male crabs .Maximum amount of protein related to group A(20-20) was 582.5 mg/lit.

Table 2. Evaluating total protein resultant from gel filteration chromatography of male blue crab

Step	Fraction	Total protein mg/L
Cephad ex- G100	Raw extract	1366
	group A	582.5
	group B	204.9
	group C	405
	group D	167

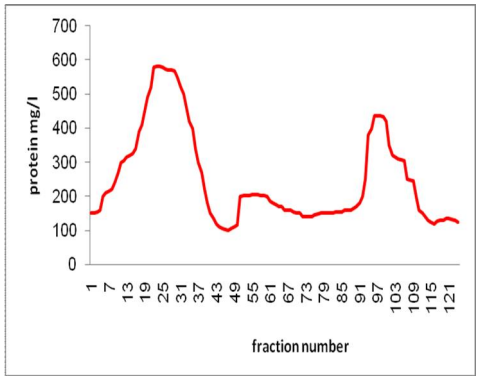

Fig 1. Results of total protein resultant from gel filteration chromatography of male

Whereas the results in female crabs showed the existance of 3 protoin groups which maximum of it was related to fractions group A(16-21) with amount of 410.9 mg/lit.

Table 3. Total protein resultant from filteration gel chromography of female

Step	Fraction	Total protein mg/L
Cephad ex- G100	Raw extract	985
	A group	410.9
	group B	365
	group C	197.5

The results of second column chromatography of group A(16-21) resultant from first column of blue crab showed 5 protein groups in males and 4 protein groups in females.

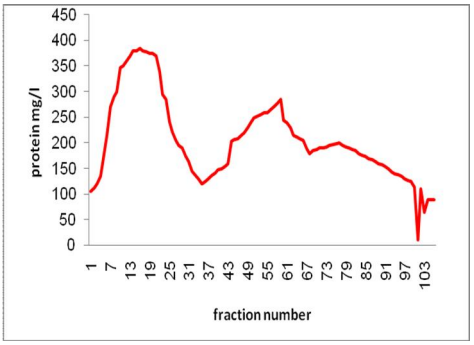

Fig 2. Results of total protein resultant from gel filterationchromatography of muscle tissue of female crab

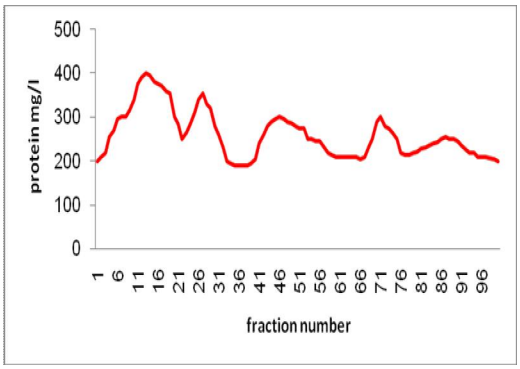

Fig 3. Results of evaluating resultant fractions of protein from group A, Ion x change chromatography in male crabs

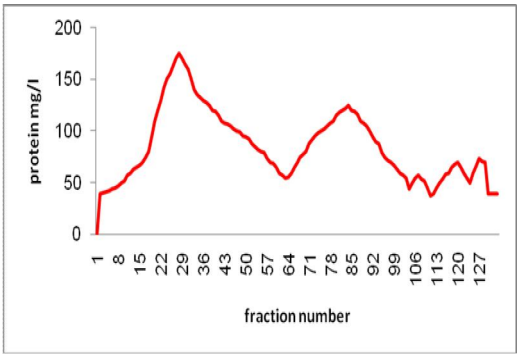

Fig 4. Results of evaluating resultant fractions of protein from group A, Ion x change hromatography in female

Results of mean tests between two sex groups of males and females had same results considering total protein amount in both groups of first and Second column fractions . In such a way that there was a meaningful differene between total protein amount in males and females at the level of 10 %

and always total protein amount was higher in males .

Also results of regression analysis showed that there is always a meaningful relation between crab's weight and total amount of protein (in both sexes) at the level of %1 which always with increase of weight , protein amount increased .

4. Discussion

Studies on *callinectes sapidus* crab showed that hypoxy (Severe decrease or drop of available amount of oxigen) in estuaries, is an important factor in producing protein and subsequently synthesis of oxidative enzymes in this variety . Also this crab is able to maintain its enzyme defense system in hypoxy situation or decreasing the level of enzyme and protein production. (kong 2003) in addition to ecological and environmental situations, biological factors also have effect on antioxidant level of animales and so as crabs . Newest researches on same of the portunidae specieses which have demorphic characteristics showed that some speciad enzyems were extracted from males' gills which are not observed in female .(mayerz and ettal 2008) .These researches belive that such differences contribute to sexual biological differences.

It seems that in greater organisms which are her maphrodite there most be significant difference in structure and type of enzyme compositons. (cleps 2009), Indeed considering this reality that males moult more than females. The animale stores a lot of protein compositons because it losts considerable amounts of water during moulting, (Thomas 1999) So it is edvident that the level of protein storage in male blue crabs will be more than females . on the other hand it can be Seen that higher mean weight of blue male crabs related to females , have a direct correlation and relationship with amount of muscle tissue. In such way that total amount of protein in males is meaningfuly more than female crabs and if this comparison is performed in maturaticn ages more diffecence can be seen .which is related to moulting stages and reproductive process of both males and females (Nelson 2008).

It is observed in this study that there was a positive and meaningful relation between wetweight and total protein in both male and female .(at the level of 0.1)In such way that with increase of weight, the total amount of protein increased considerably. Investigating other varieties of portunidae families showed that if the individuals of the variety are classified in several groups based on weight , in lighter Samples (less than 50 gr) the ratio of protein amount to chitin (Uneatable part) will decrease . where as in heavier ones (more than 100gr) ratio of protein or muscle tissue (eatable part) to uneatable part (chitin) increases significantly (juan 2006). results of this study showed that in male blue crab which have higher mean weight, there was higher total protein .Indeed in three weight classes with increasing the weight always protein amount increased.

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