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Isolation and Quantitative determination of proteins from fish scales of *Cyprinus carpio* L. and *Liza albue* In saltwater and freshwater

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Abstract

This study was conducted in the laboratories of the Faculty of Science - University of Kufa in (2014- 2015) Included extraction and isolation of deferent type of proteins from the scales of fish carps *Cyprinus carpio* and Khushani *liza albue* in salt and freshwater environments and used in the experiment peels dorsal origin. Continuously drawn to peel the fish using a systematic solution (50 m M Tris-HCl, pH 7.5)Concentration of 1M of non-protein compounds associated proteins was isolated using central ostracism and deposition of proteins from the organizer of the solution using different concentrations of ammonium sulfate$(NH_4)_2SO_4$).

The results show the presence of different protein compounds was deposited from the scale of qualitative studied fish through the use of different saturation concentrations of ammonium sulfate and achieve different and isolate the protein compounds residual saturation ratios by sulfates were classified according to the proportion saturation of Almaah gratification was quantification using a spectrophotometer deviceUV- Spectrophotometer at wavelength of 250 nm . The results show through the different types and amount of proteins in different species and studied the different growth environment.

Keywords: *Cyprinus carpio* , *liza albue*, scales, saltwater , freshwater

Introduction

Fish is an important source of iodine sources, phosphorus and calcium and vitamin A and D, it importance for the teeth, bones and blood. These vitamins found in fish liver and Fish contains a high proportion of glutamic acid, article omega-*3* fatty acids which is material necessary for the functions of the brain and nerves The presence (Which is a multi-unsaturated fatty acids found in fish fat prevents injury objects blood clots which infect the heart and brain and the importance of health and nutrition for adult fish of this study was to investigate the vehicles as a source of protein in the diet last scales fish .Common carp (*Cyprinus carpio*) belong (Fish leader Euphrates) golden land scape wonderful, and distinctive taste to Platoon Cbottiy at Cyprinidae, is one of the most important fish species in aquaculture throughout the world. Carp is an omnivorous species, feeding on plankton and benthos as well as detritus in natural conditions (Adamek et al., 2004; Mráz et al., 2012).

Fish also provide significant amounts of a number of other potentially protective components has beneficial effects on human health. These include vitamin D, vitamin B12, selenium, iodine, choline ,taurine, and fatty acids, especially highly unsaturated fatty acids (n-3 HUFA) as well as a well-balanced amino acid composition (Kris-Etherton *et al*., 2002;; Mozaffarian and Rimm, 2006; Simopoulos, , 2008; Tocher, 2010; Lund, 2013,).While water salinity in aquatic conditions represent a considerable challenge to most fish, there are additional environmental factors that interact with

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salinity to affect a fishery, such as temperature, oxygen, ammonia, selenium, arsenic and sulfide. .(Suvajdzic *et al*,2006).

Salinity affect the rat of oxygen consumption in different ways ,I.e. metabolic activities in freshwater *L.abu* (increase with increasing salinity (Ahmed,2005).

The largest freshwater species are comprised of more than 200 sex followed by 2,000 species(Chandler *et al*, 1997). It is the best-selling of all other fish, because it contains thistles, where there is no fear on the children of eating, carp, "the most important of many types and features carp rapid growth and outstanding at the rate of resistance diseases and environmental changes acute and ease of cultivation (Al-Hamed, 1971) and cover the fish scales and no bottom crust of this simple mucous material.The presence of scales will protect the fish from fungi and bacteria attached there to and in the lack of presence of up to germs article mucous hit by disease . In addition to protect the fish from stream strong and salinity of the sea and kept her skin and flesh from eating salt water . peel fish classified to four varieties depending on the components of the material, including the placoid, cosmoid, cycyloid and ctenoid has great importance in the study and determine the age of fish**.**

Physiological responses to increased salinity in fish have mainly been studied in different species of fish such as eels, salmon, and tilapia (Khalifa,1986). We have chosen the common carp (*Cyprinus carpio*) as a test organism because of its wide range of habitats**.** The common carp is a well-known example of a stenohaline fish (Kris-Etherton *et al*,2002). Although it displays some tolerance to salinity (Kliambi & Zdinak,1980, Kulijsev & Agayarova ,1984) and diluted sea water has been reported to enhance the survival, growth, and development of carp larvae (Ikoma *et al*,2003) exposure to higher levels of salinity seems to have adverse effects on carp. Growth decreases, glycogen stores are depleted, and fish become more susceptible to bacterial infections (Morzaffarian and Rimm,2006).Fish is a rich source of protein-containing amino acids such as arginine, tryptophan, including the collagen protein associated with calcium which, when removed from the protein by heating the we get gum stick y glue((Ikoma *et al*, 2003) . The eat fish in private life after the age of forty, it protects from disease.

under study.Nagai et al,(2010) Found that Fish scale was decalcified and disaggregated and then collagen was prepared by limited pepsin digestion. The yields of collagens were very high on a dry weight basis; sardine 50.9%, red sea bream 37.5% and Japanese sea bass 41.0%, respectively. This study to investigate the influence of salinity condition on the types of basic proteins in the scale carps *Cyprinus carpio* and *liza albue*.

Materials and methods.

Sample collection

Peel fish collected in this study after it was purchased from markets Local and diagnosed as a type 4 of carp fish *Cyprinus carpio* and Khushani *liuza albue* and dorsal origin and each aqueous medium separately and dried at a temperature of 40 degree using convection oven , Were crushed using a blender was configured to extract.

Alcoholic extraction and precipitation

According to methods of Ikoma *et al*, 2003) in extracting protein from the scale of fish, taking 20 Alake coral btunaih grams of powder suspended in the formal solution (50m M Tris-HCl) and pH 7 by10 ml DDT was added, EDTA to the solution to inhibit an enzyme protease was continuously move the solution in a cool place for a period of45Minutes after the expulsion of the solution was a central

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rate of 12,000 R / min and then poured the sludge and use the filtrate plus all material protamine sulfate to remove DNA and RNA .The filtrate was moving solution for 20 Minutes, after which the deposition of proteins from solution using ammonium sulfate(NH4) 2So4 According to the proportions shown in the table (1) Where used different concentrations of ammonium sulfate saturation rates which was different from the deposition of proteins according to the proportions of a solution of ammonium sulfate saturation.

Determination of total protein

Cauterizing proteins estimated in the precipitate and filtrate each separately and the two types of fish under study according to its growth environment using a spectrophotometer at a wavelength of 280 nm where they were for centrifuges in the saturated solution 3000R / min for10 Minutes and took his eye Mekdrha10Ml of solution filtrate estimated amount of the proteins and then take another sample of the sediment and the estimated amount of the proteins and given percentage for each of them as shown in the tables(1).

Statistical Analysis

Experiment carried out in accordance with the full random design (C.R.D.) according to choose less significant difference (L.S.D.) below the level of probability (0.05) (Al-rawi and Khalaf Allaha, 2000).

Results and discussion

The results shown in the table (1) The present of significant differences in the amount of protein extracted from the peels Khushani Fish *liuza albue* developing in the freshwater environment depending on the type of the sample studied and the percentage of saturation with ammonium sulphate . Filterate solution has achieved the first sample and by the satisfaction of (30%) the highest amount of precipitated proteins compared with the rest of the other transactions in accordance with the saturation rates have reached the amount of protein in the sample (218.7)mg / ml and differed significantly from the rest of the transactions and has excelled in a significant amount of protein in the sediment of the same kind of saturation ratio, which amounted to(197) mg / ml, as for the level of55% Saturation of the amount of protein decreased and reached in filtrate about (49.2)Mg / ml compared to the amount of protein in the serene first treatment differed significantly for the treatment of Sediment at the same level of saturation, which amounted to(11.7)mg / ml, which in turn differed significantly from the previous treatment and the same level of saturation.The same table shows a clear decline in the amount of protein at a level of saturation(90%) Which amounted in filtrate(11.1)mg / ml and differed significantly from the rest of the transactions made while Sediment less amount of protein reached(3.3)mg / ml and showed significant differences with the rest of the transaction.

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Table (1) Protein content mg / ml in samples dorsal cortices of *l.albue* in the freshwater environment

proteins Content	Protein in the extract mg /ml	Protein in the sample Mg / ml	Sample quantity of Sediment µl	The amount of mitigation DW	The amount of salt g / l	The level of saturation	Sample type
72.427	218.7	0.2	10	990	8.8	30%	filtrate
84.494	197	9.2	5	995	8.8		Sediment
22.496	49.2	10.8	20	280	8.1	55%	filtrate
3.939	11.7	2.8	10	290	8.1		Sediment
5.075	11.1	1.1	20	280	13.2	90%	filtrate
1.111	3.3	0.8	30	270	13.2		Sediment

According to results shown in the table (2) An increase in the amount of proteins in the dorsal cortices of ***liza albue*** samples .grown in salt water environment and the existence of significant differences in the amount of protein derived from them, depending on the type of the sample studied and the percentage of saturation with ammonium sulphate, the sludge has achieved the first sample and by the satisfaction of(30%)The highest amount of precipitated proteins compared with the rest of the other transactions in accordance with the saturation rates have reached the amount of protein in the sample filtrate (315)mg / ml and differed significantly from the rest of the transactions and has excelled in a significant amount of protein in the sediment of the same kind of saturation ratio, which amounted to(291)mg / ml, as for the level of(55%) Saturation of the amount of protein decreased and reached in filtrate(55.1)mg / ml compared to the amount of protein in the serene first treatment differed significantly for the treatment of sludge at the same level of saturation, which amounted to(231)mg / ml, which in turn differed significantly from the previous treatment and the same level of saturation.The same table shows a clear decline in the amount of protein at a level of saturation(90%) Which amounted in filtrate(6.1)mg / ml and differed significantly from the rest of the transactions made while sludge less amount of protein reached(3)mg / ml and showed significant differences with the rest of the transaction.

Table (2) Protein content mg / ml in samples dorsal cortices of *l. albue* in salt water environment

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Proteins Content	Protein in the extract mg / ml	Protein in the sample mg / ml	Sample quantity of Sediment µl	The amount of mitigation DW	The amount of salt g / l	The level of saturation	Sample type
72.427	315	0.4	10	990	8.8	30%	filtrate
94.494	291	22.1	5	995	8.8		Sediment
22.496	55.1	11.8	20	280	8.1	55%	filtrate
3.939	31.2	2.6	10	290	8.1		Sediment
5.075	6.1	2.1	20	280	13.2	90%	filtrate
1.111	3	1.1	30	270	13.2		Sediment

The results (Table 3) the existence of significant differences in the amount of protein extracted from the dorsal cortices of carp type *Cyprinuscarpio* developing in the freshwater environment depending on the type of the sample studied and the percentage of saturation with ammonium sulphate, gave the first sludge sample and by the satisfaction of(30%)The highest amount of precipitated proteins compared with the rest of the other transactions in accordance with the saturation rates have reached the amount of protein in the sample of sludge(388)mg / ml and differed significantly from the rest of the transactions and has excelled in a significant amount of protein in the serene and for the same kind of saturation ratio, which amounted to(257)mg / ml, as for the level of(55%) Saturation of the amount of protein decreased and reached in filtrate (65.2)mg / ml compared to the amount of protein in the first sludge treatment differed significantly for the treatment of sludge at the same level of saturation, which amounted to(22.7)mg / ml, which in turn differed significantly from the previous treatment and the same level of saturation.The same table shows a clear decline in the amount of protein at a level of saturation(90%) Which amounted in filtrate(15.1)mg / ml and differed significantly from the rest of the transactions made while sludge less amount of protein reached(3.3)mg / ml and showed significant differences with the rest of the transaction**.**

Table (3) Protein content mg / ml in samples dorsal cortices of carp type *C. carpio* developing freshwater

proteins	protein in	protein	The	The	The	The level of	Sample

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content	the extract mg / ml	in the sample mg / ml	sample amount of Sediment µl	amount of mitigation DW	amount of salt g / l	saturation	Type
82.891	257	1.8	10	990	8.8	30%	filtrate
91.555	368	24.2	5	995	8.8		Sediment
58.674	65.2	18.8	20	280	8.1	55%	filtrate
5.939	22.7	4.2	10	290	8.1		Sediment
3.132	15.1	3.1	20	280	13.2	90%	filtrate
2.334	6	1.8	30	270	13.2		Sediment

Regarding the dorsal cortices results indicate in the table (4) the existence of significant differences in the amount of protein extracted from carp developing in saltwater environment depending on the type of the sample studied and the proportion of saturation with ammonium sulphate, gave the first Sediment sample and by the satisfaction of(30%).The highest amount of precipitated proteins compared with the rest of the other transactions in accordance with the saturation rates have reached the amount of protein in the sample of sludge(345)mg / ml and differed significantly from the rest of the transactions and has excelled in a significant amount of protein in the serene and for the same kind of saturation ratio, which amounted to(260)mg / ml, as for the level of(55%)Saturation of the amount of protein decreased and reached in filtrate(76.5)mg / ml compared to the amount of protein in the first sludge treatment differed significantly for the treatment of sludge at the same level of saturation, which amounted to (43.7)mg / ml, which in turn differed significantly from the previous treatment and the same level of saturation.The same table shows a clear decline in the amount of protein at a level of saturation(90%), Which amounted in filtrate(13.1)mg / ml and differed significantly from the rest of the transactions made while sludge less amount of protein reached(4) mg / ml and showed significant differences with the rest of the transaction.

Table (4) the amount of protein mg / ml in samples of dorsal cortices for carp type *C. carpio* developing in saltwater

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proteins content	protein in the extract mg / ml	protein in the sample Mg / ml	The sample amount of Sediment µl	The amount of mitigation DW	The amount of salt g / l	The level of saturation	Sample Type
83.1 91	260	2.4	10	990	8.8	30%	filtrate
94.555	385	26.1	5	995	8.8		Sediment
68.174	76.5	17.8	20	280	8.1	55%	filtrate
8.223	43.7	4.6	10	290	8.1		Sediment
3.9 32	13.1	2.8	20	280	13.2	90%	filtrate
1.334	4	6.1	30	270	13.2		Sediment

L.S.D(0.05)=6.812

Results in table(4) demonstrated the presence of varying amounts of proteins with significant differences were apparent between the dorsal cortices of carp fish(*C. carpio)* and *l. Albue* variety with different kinds of fish and environments condition of habitat .The reason for this may be due to functional divergence of these peels for the same type of fish, which varies depending on the growth environment where the advantage peels dorsal defensive function against the water currents and various pathological causes the presence of fungi and bacteria attack (Ahmed,1986).

In addition to protecting the fish from the strong currents and salinity of the sea and kept her skin and flesh from eating salted water ,this requires the presence of more rigidity in the structure of this kind of peels depending on location as well as the function of giving the distinctive color of this type of fish , which is one of the most important causes is the presence of certain types of these proteins .

About this results shown in the tables above superiority of the carp developing Carp fish scales show in salt water upon in the total amount of proteins on the quantity of the *l. albue* scales developing fish in the same environment and the quantity in the peels dorsal to fish the same type developing in fresh water while outperformed peels dorsal carp developing in fresh water.

While outperformed peels dorsal carp in fresh water superior in the amount of proteins, quantities in the crust dorsal developing in the environment itself to peel *l. albue* Fish recorded significant differences were evident in the amount of proteins between peels dorsal carp compared with peels dorsal *l. albue* fish , and achieve the same moral differences in total amount of proteins between the dorsal scales of the fish studied , which is due to the genetic nature of these fish.As well as the reason could be due to the nature of the circumstances surrounding the growth of fish , affecting the environment salt in their adaptation to resist the stress with the existence of excess salts in the environment Fish medium.

Several study reported that the ability of common carp and *l. albue* to different salt concentration (Kilambi and Zdinak 1980; Clover and Smith (1987; Al-Shami 2006). Common carp and *l. albue* posses an tolerance to salt water in different ways (gabber et al,2007).

The study by Al-Faiz(2009) conducted to determine the effect of direct transportation of the common carp (*Cyprinus carpio*) fingerlings to salt solution reveled that the high mortality 100 % occurs during the first 24 hours at high salt concentrations solution.

Acid-soluble collagen (ASC) and pepsin-solubilized collagen (PSC) were isolated from the bones and scales of black drum (*Pogoniacromis*) and sheepsheadseabream (*Archosargusprobatocephalus*) caught in the Gulf of Mexico(Ogawa *et al* 2004).

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