

The use of electrophoresis technique to isolation of myofibrillars proteins from common and silver carp treated with different preservation and cooking methods

Hala Yhya Essa Al-Robeay ¹, Khadeeja Sadiq Jaffer Al- Hussainy ^{1*}

Food Sciences, College of Agriculture, University of Basrah, IRAQ Corresponding author: khadeeja_jaffer@yahoo.com

Abstract

The current study dealt with the use of methods of preserving (freezing and salting with drying) and cooking methods (broiling, cooking in broth and frying) and studying their effect on myofibrillars proteins separated from the meat of common and silver carp and diagnosed with electricrelay technology, the study was conducted during the period (10/2018) - (1 / 2020). The results were as follows:

Myofibrillars proteins varied in the two fish types, they are indicated by the number of protein bands separated from them according to the different fish and protein types. This indicates that the separated myofibrillars proteins contain a similar percentage of different types of proteins, but they are not equal in their density and intensity.

The results showed the presence of 5 protein packs for the myofibrillars proteins of fresh, frozen, dried, roasted, fried and fried common carp fish, and 9 protein bands for the common carp cooked in broth, while 6 protein packs appeared for the myofibrillars proteins of fresh and grilled silver carp fish and 5 protein packs for the myofibrillars protein of the silver carp fish frozen and fried, 7 protein packs for the myofibrillars protein soft the dried salted silver carp, and 10 protein packs for the silver cooked broth, respectively.

Keywords: cooking methods, preservation methods, myofibrillars proteins, common carp, silver carp, Electrophoresis Technique

Al-Robeay HE and Al- Hussainy KS (2020) The use of electrophoresis technique to isolation of myofibrillars proteins from common and silver carp treated with different preservation and cooking methods. Eurasia J Biosci 14: 2247-2255.

© 2020 Al-Robeay and Al- Hussainy

This is an open-access article distributed under the terms of the Creative Commons Attribution License.

INTRODUCTION

The electrophoresis technique was one of the approved techniques for distinguishing proteins from their different sources. The principle of separation of proteins depends on the transport of protein molecules charged during the gel under the influence of volume and charge if the electric current passes into the used device, which varies under specific conditions in terms of its mass and its ability to cross through the supporting surface Porosity; The larger molecules (more molecular weight) cannot pass through the small pores of the gel, which gives an additional separation of the molecules based on their molecular size, reinforcing the feature of electrophoresis using polyacryl amide gel, which has pore sizes similar to the sizes of proteins, and the use of acrylamide helps in giving the gel A more open porous structure (Hantoush, 1998; Adda, et al, 2016).

Montowska and Pospiech (2007) explain that electrophoresis was used to determine the flesh type for different classes of animals and relies on protein separation in an electric field after extracting it from muscle tissue. The differences between the types of fish depend on the number of beams that appear on the surface of the protein-preserving gel, the distance they travel from the starting point, or the concentration of proteins in different tissues; when its concentration is high, its pigmented is darker and it is lighter when its concentration is low.

The results of the electrophoresis on acrylamide gel conducted by Al-Hamdani et al. (2016) showed the presence of (10-11) protein bands and high molecular weights and the appearance of contrasting bands in their density while the partial weights of the patient fish were within the limits of the standard proteins, and when diagnosing the proteins of the Sapoor fishes fall five proteins (Gelsolin, C.protein, M1/M2, Fimbrin, actin), And when comparing Sapoor fish proteins with other fish proteins, including *Liza abu* ,Common carp *Cyprinus carpio Ilisha megaloptera*, *Chirocentrus dorab*,

Received: September 2019 Accepted: April 2020 Printed: July 2020 EurAsian Journal of BioSciences 14: 2247-2255 (2020)

Cynoglossus bilineatus, Luciobarbus xanthopterus, Arabibarbus grypus, Nematalosa nasus and Cynoglossus arel The fishes from the same area and the nearby rivers were closer to bonds with fish proteins Chirocentrus dorab, Cynoglossus arel and Nematalosa at the probability level ($P \le 0.05$) While the association of patient fish proteins was very close to each other, the highest link was between proteins M1/M2 and proteins C. protein. Whereas, the lowest binding rate was between two proteins Actine and gelsolin on 0.819.

Aim of the Research

The current study aims at the use of the electrophoresis technique to diagnose myofibrillars proteins in the meat of common and silver carp.

MATERIALS AND METHODS

Fish Samples: Samples of fresh (common carp (samty) and silver carp (dukhan)) fish were brought from the local markets of Basrah city, kept in refrigerated folders. After arriving at the laboratory, a section of them was kept frozen for a period of four months, as well as conducting salting by 10 % and then drying for a period of four months and other samples are used in different cooking methods such as barbecue for fish samples at 300 °C and for 45 minutes and cooked broth at 100 °C for 45 minutes.

A- Extraction of Muscular Proteins (myofibril):

Muscle tissue proteins are separated, and this is done by adding a sufficient amount and with a specific concentration of salts that are usually ammonium sulfate or sodium chloride or sodium chloride or potassium. Many proteins are deposited, which are separated by centrifugation, and another amount of salt which can be added to deposit the protein to be purified (Al-Azzawi, 1996) and Pivoted method of Huda et al. (1994) in muscle fiber proteins separation.

- 1- The meat was cut and finely chopped.
- 2- 200 g of minced meat was taken.
- 3- The minced meat is mixed with a NaCl 1% saline solution in a 2: 1 ratio (meat: a saline solution) with an electric mixer.
- 4- Centrifuge is discarded at a velocity of 5,000 cycles / minute for half an hour, and the precipitate represents the muscle fiber proteins.
- 5- The sediment was emptied by the Vacum Oven at 55 °C.
- 6- After drying, the precipitate was ground with a ceramic mortar to soften it, and then it was kept in the refrigerator at 7 ± 2 °C until use.

B- Fractionation of myofiblers proteins separated from fish meat by electrophoresis and by using a polyacrylamide gel:

The electrophoresis method was adopted in a polyacrylamide gel and in the absence of denatured agents according to the Laemmli (1970) method which

is described by Garfin (1990) in the fractionation of myofibril proteins with some modifications, and the experiment was performed in the genetic engineering laboratory at the Faculty of Agriculture / University of Basrah.

1- The materials and methods used:

Materials: Myofibril proteins which were used and extracted from the fish.

Sample preparation: Myofibrillars proteins were dried and ground with ceramic mortar and sifted with a fine-hole sieve, then 1g of these dried, crushed and ground proteins were taken and 5 mL of Tris base solution was added after modifying the pH to 7.2 for the dried proteins, a one-hour glass stick was used for each sample and the samples were frozen until the electrical transfer occured.

Preparing the solutions

1- Solution No. (1) Acryl amide solution: dissolve 37 g of acrylamide and 0.925 g of (mathlene bis acrylamide) MBA prepared from the English company BDH in 60 ml distilled water, and complete the volume to 100, then filter the solution. During Watman filter paper No.1, it was kept in a dark bottle at 7 $^{\circ}$ C.

2- Solution No. (2) Tris (Hydroxy methyl) methyl amine: was prepared by dissolving 9,070 g of Tris (Hydroxy methyl) methyl amine, 2 g of citric acid and 20 g of TEMED (N, N, N, N-tetra methyl ethylene diamine) in water. Then distilled the volume to 100 ml.

3- Solution No. (3) ammonium persulfate solution (APS): 0.045 mg of ammonium persulphate was dissolved in 10 ml of distilled water and the volume was supplemented to 30 ml (prepared just before the experiment).

4- Solution No. (4) concentration gel (buffer solution for stacking gel Stacking gel buffer pH = 6.8): was prepared by mixing 1.5 ml of solution No. (1) and 1.5 ml of solution (2) and 9 ml of solution No. (3).

5- Solution No. (5) buffer solution: Electrode buffer (pH) = 9.5: was prepared by dissolving 10 g of base tris and 1.75 g of boric acid with an amount of distilled water and then the volume was completed to 1.25 liters.

6- Solution No. (6) solution staining solution: Prepared by dissolving 1g of blue kumasi tincture in 200 ml of the 7% solution of acetic acid (volume: volume).

7- Solution No. (7) Solution Distaining solution: It was prepared with a concentration of 7% snowy acetic acid.

8- Solution No. (8) Sample solution Stock sample buffer: 5 microliter of samples were taken and 15 microliter of bromophenol blue dye was added (size became 20 microliter) and 5ml of it was taken for injection purpose. The purpose of adding the dye is a guide to the movement of samples and for the consistency of the sample and not Exit while adding buffer solution to the electrodes.

Preparing the gel: Prepare the gel by mixing 10 ml of solution No. (1) with 7.5 ml of solution No. (2), 7.5 ml of solution No. (3) and 6 ml of distilled water.

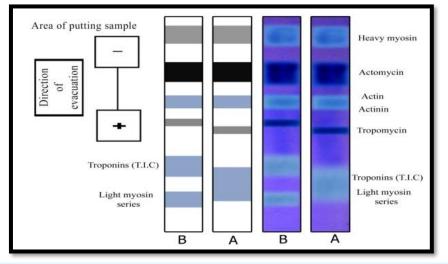


Fig. 1. Electrophoresis of the myofibrillars proteins of fish (A) fresh common carp and (B) fresh silver carp

Jelly pouring process: Pouring the gel formed from the previous quantities directly into the Chamber between the two glass slides and with special care to prevent the formation of air bubbles 10×10 mm by syringe with a capacity of 10 ml, and left them until the solidification and after completion of hardening up to 20 -25 minutes pour the concentrated gel (solution No. 4) and apply the comb before hardening the gel, and the gel was left to solidify for 30 minutes after which the comb was carefully lifted to prevent deformation in the gel.

The process of injecting the samples: The samples were glazed (solution No. 8) by using a microsyringe measuring 5 microliter, after which the depot was placed in an inverted manner (the slide is turned over) in the electrical relay device to contact the reservoir opening of the electrode reader (electrolytic solution) or solution number (5) During deportation.

Operation of the device: The Maxi Vertical Electrophoresis type electric relay device imported from the British company Cleaver Ltd Scientific was used for 24 samples.

After the depot representing the negative electrode (cathode) and the buffer solution (No. 5) representing the positive electrode (anode) were placed, the electrical circuit connected the relay device and the power supply, the outer cover of the device was installed and the compressors were placed, and the power supply was powered to 90 volts and after Half-hour passage raises voltages from 225 to 250 volts for 4-5 hours to obtain a complete separation of the model's components until the blue bromophenol beam reaches the end of the gel (noting that a one-hour pre-pass gel process is observed before modeling).

Removing the gel: The separation gel was removed from the glass plate by pushing an amount of water by syringe on the inner wall of the tubes and carefully to avoid tearing the gel. The gel was placed in the pigment solution which is solution n (6) for one night, and the dye was removed from the gel using a solution 7% glacial acetic acid and solution (7) to wash the gel several times until protein bands appear clear, and the gel is preserved with distilled water.

RESULTS

Electrophoresis of Common and Silver Carp Sample Proteins

Figs. 1-6 deportation results are shown Electrophoresis for myofibrillars proteins for common and silver carp were preserved with different preservation methods and also were cooked with different cooking methods.

Electrophoresis of myofibrillars proteins from fresh samples of common and silver carp

The results showed that there are 5 and 6 protein bands for the myofibrillars proteins of common carp and fresh silver carp respectively, and these bunds were varied in intensity in the different gel regions, and their molecular weights ranged between heavy and light starting from the upper part of the gel to its end.

In myofibrillars proteins of fresh common carp fish, a dark and wide protein bund was observed in the top of the gel. This band represented heavy myosin, followed by a dark and wide band that was the complex of actomycin and a pale band less width than it belongs attributed to the actinine, and then a thin, pale band that attributed to tropomycin and in the end of jelly appeared a very pale and vary wide band which was proteinbounds convergent in molecular weight that may comprise actin, T-troponin, I- troponin and C-troponin respectively, and the remaining actin and myosin residues (Fig. 1A). As for the myofibrillars proteins of fresh silver carp, protein bands appeared in different density and thickness bodies, the first of which was a dense and wide band at the top of the gel that belongs to the heavy myosin chain, then a very thick and dark band caused by the combination of myosin and actin to form the actomycin complex, then a medium-width and pale band of the actinin protein and another pale band after which it attributed to tropomycin, It was followed by two clear and pale bands, the first band indicated to Ttroponin, I-Troponin, C-Troponin and actin residues respectively, and the last one indicated to the light myosin band (**Fig. 1B**).

These results are close to what Focant et al. (2000) When they separated the fibrils of the Turbot fish by electrophoresis on the SDS-PAGE gel, bat they differed from the results of the study found by Al-AbdNabi (2003) in the number of bands obtained when they separated the fibrils of the carp fish by electrophoresis by SDS-PAGE, which showed that there were 13 protein bands that varied in intensity, and their molecular weights ranged from the top end of the gel to its end between (112200-17400) Dalton. This was in addition to the presence of a protein band on top of the gel that did not enter its pores due to its large molecular weight, and she noticed the existence of two protein bands, The first is pale and the second dense band at the top of the gel and they had two molecular weights (112200 and 109600) Dalton respectively, and the dense band attributed to one of the two heavy chains of myosin, and in the middle of the gel a dense band with a molecular weight of 42700 Dalton appeared and attributed to the actin protein, the band that came after the actin band attributed to a protein tropomycin, which has a molecular weight of 29,500 Daltons, and due to the convergence of the molecular weights of the troponins proteins (T, I and C) and the three light myosin chains (MLC-1, MLC-2 and MLC-3), the remainder of the protein bands may attributed to some of them separately, or these proteins were bound together in these bands, the molecular weights of these bands ranged between (17400-25100) Dalton. The gel contained acidic and basic peptides weighing less than 14.4 kDa, as reported by Marcone et al. (2002). Molecular weights ranging between (20-27 and 30-39) kDa are short peptides.

This variation in the number of separated bands confirms that there were clear differences in this type of protein and consequently between fish types, and these differences may be due to the nature of living for each type of fish, such as the type of food and the environment in which they live and so on.

When comparing these results with the findings of Pineiro et al. (1999) In their diagnostic study of flat fish species, after separating their water-soluble proteins using two-dimensional electrophoresis, and the number of diagnosed proteins in each of them reached 11, 14, 16 and 17 proteins, these results are very far from them.

Electrophoresis of myofibrillars proteins from frozen samples of common and silver carp

The results showed that there were 5 protein bands from myofibrillars proteins for frozen samples of

common carp and silver carp. These bands varied in intensity in the different gel regions, and their molecular weights ranged between heavy and light starting from the upper end of the gel until its end.

In the myofibrillars proteins from frozen samples of common carp and silver carp, it was noted that the first and second bands were dark and wide, which belonged to the actin and actinin protein chain, then followed by a pale band that seemed a little thicker attributed to the residues of actomycin and had gone further in the gel due to its light weight, followed by a pale and thin protein band. It attributed to the tropomycin proteins and the last band was wide and dark attributed to the troponins proteins (T, I, C) and the two light myosin chains, and the disappearance of the myosin protein from the frozen samples was observed for four months (**Fig. 2A**) and (**Fig. 2B**).

The current results were supported by a study conducted by Kasapis (2009), which stated that myosin in particular was more susceptible to denterzation, and at the same time the actin maintained its natural form without significant change, as Benjakul et al. (1997) showed that the actin protein in Pacific whiting muscles was more resistant to protein decomposition than myosin MHC.

The current results were consistent with the findings of the Subbaiah et al. (2015) who studied histological changes and the extent of protein degradation during frozen storage at -18 °C for 150 days for Nile tilapia (Oreochromis niloticus), and the researcher stated that during this period, soluble proteins in salt decreased and the solubility of proteins decreased significantly and noteworthy during the duration of frozen storage, Electrophoresis was carried out using sodium dodecyl sulfate polyacrylamide gel, and it was found that the heavy myosin and actin chains were major components of the protein chain, and no visible changes were observed in the heavy myosin chain (MHC; 212 kDa) until today 90 of the duration frozen storage However, it was found that the thickness of the bands began to decrease after 90 days, and the actin (45 kDa) appeared predominantly and no change was observed in it during all freezing periods, indicating that the actinin was more resistant to protein degradation, and a change occurred in the band pattern in 120 and 150 day of storage, and several lighter beams in gel weighing 45 kDa and 29 kDa appeared during this period, moreover, the density of the bands began to decrease with the freezing period, and this may be due to the breakdown of proteins with high molecular weights that emerged after 90 days of storage, Liu et al. (2010) referred to similar results in Oreochromis niloticus, and there was a decrease in the severity of the beams with an increase in the freezing period of the cod muscles (Ohnishi and Rodger, 1980), as Chummar et al. (2004) noted the same decrease in the muscles of the frozen roho and mackerel fish.

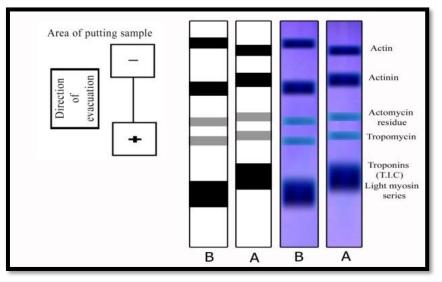


Fig. 2. Electrophoresis of the protein fibers of fish (A) frozen common carp and (B) frozen silver carp

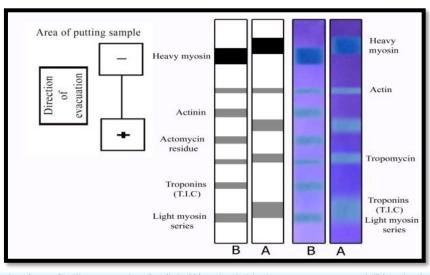


Fig. 3. Electrophoresis of myofibrillars proteins for fish (A) salted dried common carp and (B) salted dried silver carp

Lu et al. (2019) when studying big-head carp, the myosin (kDa) bands were thinner in week 8 and 16 than in the first week at freeze -12 °C. In particular, the density of the myosin MHC and actin bands (42 kDa), tropomycin (36 kDa) and T-troponin (35kDa) decreased significantly at week 16, and the bands of myosin, actin, tropomycin and Troponin proteins did not show any apparent changes during frozen storage at -28 °C, and likewise, Saeed and Howell (2002) found there was a significant decrease in the density of the MHC bands in minced mackerel stored at -10 °C for 4 weeks but the about fish stored at (-20 and -30)°C, MHC beams were still evident, and stated that the density of MHC, actin, tropomycin and T- troponin beams in week 16 at -12 °C was much lower than that of these proteins at -28 °C, and many studies have shown the sensitivity of muscle fibers proteins to protein degradation with cathsinase enzymes such as MHC, actin, tropomycin and troponin, and that the degradation of muscle fibers protein at -12 °C was not only due to the freezing process, but due to the protein degradation of the proteins of these samples which was more apparent than that at -28 °C.

Electrophoresis of myofibrillars proteins from salted dried samples of common and silver carp

Five bands of myofibrillars proteins appeared for the salted dried carp. It was noted that all of the bands seemed pale and differed in the amount of thickness, the first band belonging to the heavy myosin chain, then followed by three pale and thin protein bands located at different distances from each other in the jelly and indicate to the actinin proteins and a band actin and residual actomycin also that anthor band indicate to the tropomycin and the last band attributed to T-troponin, I-troponin, C -troponin and two light myosin bands (**Fig. 3A**).

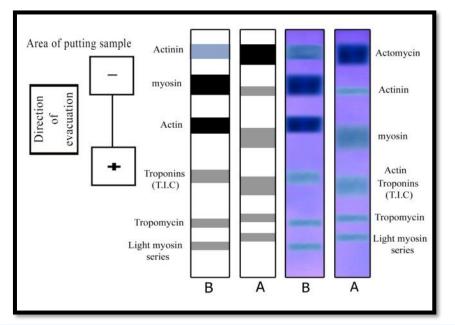


Fig. 4. Electrophoresis of the myofibrillars proteins of fish (A) grilled common carp and (B) grilled silver carp

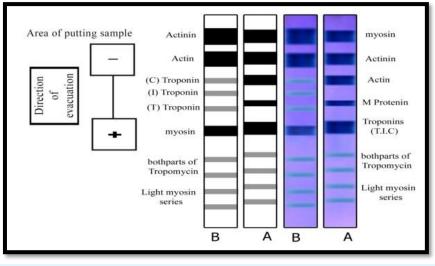


Fig. 5. Electrophoresis of myofibrillars proteins for fish (A) common carp cooked in broth and (B) silver carp cooked in broth

When separating the myofibrillars proteins of the dried salted silver carp fish, 7 protein bands appeared and it was observed that these bands were in a pale form differentiated in thickness the first of which was a heavy myosin band, then two thin, pale and clear bands attributed to the actinin and the actin respectively, followed by a pale seemed a bandthat attributed to the residual actomycin walked further in the gel due to her light weight, and merged with five thin, pale bands belonging to the T-, I- C- Troponin due to the convergence of their molecular weights and tropomycin with the two low myosin chains (**Fig. 5B**).

The results of the current study were compatible with the study of the Al-Abdul Nabi (2003) in which it dealt with the electrical relay of salt-dissolved proteins for carp fish, which showed the presence of 6 protein bands whose molecular weights ranged from the upper area of the gel to its end between (112200 - 12900) Dalton in the case of carp fish with the presence of a protein band at the top of the gel did not enter the gel pores due to its large molecular weight, and these protein bands included most of the major fibrin proteins and some sarcoplasm proteins dissolved in salt.

When comparing these results with the other studies results, we find that the salt dissolved protein extract for carp has given more protein bands than the salt dissolved protein extract for cod fish, which was determined by Rodger et al. (1980) in their study of this type of protein.

Electrophoresis of myofibrillars proteins from grilled samples of common and silver carp

Consequently, 5 protein bands appeared in the myofibrillars proteins of grilled common carp, the first of which at the top of the gel was a huge and very dense protein band that seemed to have a very large molecular weight that made it unable to get off more in the gel and attributed to the complex of actomycin, while a light and thin band emerged that was mostly actinin, Then a dense and wide band was the heavy myosin chain, followed by a light and wide band attributed to the actin protein, and it merges with three bands, one pale and the other two thin, and these bands indicate to the (T-, I-, C-) troponins and at the bottom of the gel appeared two light myosin chains (**Fig. 4A**).

The study showed the presence of 6 protein bands in the myofibrillars proteins of the grilled silver carp fish, and at the top of the gel appeared a pale and thin protein band attributed to the actinin and a pale second band that looks wider is the myosin chain and the lower band attributed to the actin, and we note that the complex of actomycin has separated into the protein myosin and actin in jellies, and then a light and wide band appeared, mostly (T-, I-, C-) troponins respectively, fused with pale bands consisting of the two parts tropomycin, and two light myosin bands appeared at the bottom of the gel (**Fig. 4B**).

Electrophoresis of myofibrillars proteins from cooked in broth samples of common and silver carp

Concerning the myofibrillars proteins of common carp cooked in broth, 9 protein bands appeared. At the top of the gel appeared two dense protein bands and two consecutive thin chains, heavy myosin and actinin chains, and then came a dense and thin band of actin, and a dense and thin band of M protein also a pale and thin protein band attributed to (T-, I-, C-) troponins and we notice that the complex of actomycin has disappeared from the gel, followed by two pale and thin bands that were the two parts of the tropomycin, then in the lower part of the gel, two light myosin chains appeared (**Fig. 5A**).

It was also noted from **Fig. 5B**, the appearance of 10 bands that attributed to the protein fibers separated from the silver carp cooked in broth form, was represented by the presence of two dense and wide protein bands, the first attributed to the actinin chains and the second attributed to the actin, followed by three pale and thin bands, attributed to (T-, I-, C-) troponins respectively, the following dense and wide band attributed to one of the two heavy myosin chains, and in the middle of the gel appeared two pale and thin bands, which were the dividing parts of the tropomycin split, and in the lower part of the gel appeared two weak and pale bands were respectively two myosin light chains. Qixing et al. (2016) search different protein components of the muscles of

big- head carp fish using electrophoresis on the SDS-PAGE gel after being heated at different temperatures, and indicated a decrease of 50 °C in the myosin and actin proteins, followed by a slow decrease of 50-80 °C, and then a slight change occurred at preheating to above 80 °C, the soluble proteins in salt were separated by SDSPAGE gel and the proteins were distributed in raw materials over a wide range of molecular weights over 200 kD and about 43 kD and initially identified as the heavy chain of myosin and actin respectively, and it was observed get down significant in the protein at 40 °C, indicating that there were no denaturation proteins soluble in salt at this temperature, and when the base temperature rose to 50 °C, protein bands with weights greater than 66.4 kDa and less than 29kDa were appeared, While the actin band (43 kDa) became significantly less dense, and upon heating above 80 °C, bands of proteins with simple molecular weights of 37-44 kDa remained present in the gel, which might be heat-resistant T-troponin (37 kDa).

Electrophoresis of myofibrillars proteins from fried samples of common and silver carp

In the myofibrillars proteins from fried of common carp and silver carp, 5 protein bands appeared, as a pale band it was actinin found in the upper part of the gel, and the two bands that came after it were a light band that indicated actin protein and a band that attributed to the residues of actomycin and then two pale bands the first was the (T-, I-, C-) troponins, and the wider one is protein M, and then the light myosin bands at the bottom of the gel (**Fig. 6A** and **Fig. 6B**).

When conducting electrical relay by Hu et al. (2017) using acryl amide gel for fresh cultured sturgeon proteins (Acipenser gueldenstaedtii), as in this study the fish slices were cooked using five different methods (boiling, steam, microwave, roasting and frying) to an internal temperature of approximately 85 ° C, bands of different proteins appeared in degrees varying from endurance to heat, the bands were distinguished in fresh, boiled, steamed and microwave oven samples, while the roasted and fried sample showed bands with a molecular weight of about 50 kD. Most surprising things during the changes in these bands were the loss of the heavy myosin band from the top of gel, and the mycin band found in fresh and boiled and cooked sample steam but weakened in the samples cooked on microwave oven then shrunk in the samples roasted and fried, as the thermal treatment of cruel causes the loss of protein mycin because the proteins are heat-resistant, according to what researchers mentioned it.

It was worth noting that the tropomycin protein band and two light myosin chains appeared on a single line in almost all gels and that they differed in their thickness. Inferred from shapes to existed similarities on the number of bands were separated and its location on all of samples of proteins which indicate to that myofibrillars

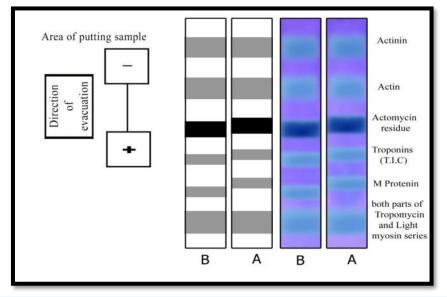


Fig. 6. Electrophoresis of the myofibrillars proteins of fish (A) fried common carp and (B) fried silver carp

proteins contained a convergent rate of vary types of proteins.

The difference, however, differed in their intensity, and the greater the intensity of the beam, this indicates an increase in the protein concentration. And the different of the beam return to the quality and quantity of amino acids present in the extracted materials and on the nature of the bonds between the peptide chains on the one hand and the amino acids on the other hand, these results were consistent with Takahashi et al. (1988), as they showed that the bands vary with different protein sources, and this variation was due to differences in the distribution of molecular weights.

The results were consistent with Habib (2010), who observed in her study of muscle fibers proteins separated from the lateral muscles of marine and river fish(sea water and Shatt Al Arab water) *Tenualosa ilisha* and *Nematalosa nasus* using SDS-PAGE electrophoresis and polyacrylamide gel (1.2 %), that the protein bands the extracts differed in the intensity and their movement according to the difference in their weights. The lightest protein bands (with the smallest weight) can pass quickly through the pores of the gel and were under the separation gel, while the heavier protein bands (with the largest weight) are seen above the separation gel, and were able to separate 9 bands from *Tenualosa ilisha* fish and 7 protein bands from *Nematalosa nasus* of filamentous marine waters and the Shatt al-Arab.

CONCLUSION

In conclusion, difference number of proteins bands were noticed when separation myofibrillars proteins by using electrophoresis technique following kinds of proteins, kinds of meats fishes.whereas the number of proteins bands ranging from 5 -9.

REFERENCES

- Adda A, Mohammed C, Djamel S, Abdelkader B, Khalladi M (2016). Remote Sensing of Evapotranspiration in a Southern Mediterranean Forest. Application to Bissa Forest, Algeria. The International Journal of Biotechnology, 5(3): 35-44.
- Al-AbdNabi SHA (2003). Separation and diagnosis of proteins of some fish using gel filtration chromatography and electrical relay and study of their functional characteristics, Master Thesis. College of Agriculture Basra University: 70 p.
- Al-Azzawi HTH (1996). Separating muscle proteins and using them in preparing some foods. Master Thesis, College of Agriculture University of Baghdad. 70 p.
- Al-Hamdani QH, Al-Shammari AG, Wasser AT, Yaqoub AK (2016). A comparative study of the chemical synthesis and separated proteins of patient fish from Abi Al-Khaseeb and Fao regions *Tenualosa ilisha* (Hamilton, 1822) Basra Agricultural Sciences, 29 (1): 274-257.
- Benjakul S, Seymour T A, Morrissey M T, and A H (1997). Physicochemical changes in Pacific whiting muscle proteins during iced storage. J. Food Sci., 62: 729-733.

- Chummar B, Nambudri D D, and Sherief P M (2004). Studies on electrophoretic pattern of proteins in frozen stored fish. Fish. Techn. 41: 109-116.
- Focant B, Collin S, Vandewall P, and Huriaux F (2000). Expression of myofibrillar proteins and parvalbumins isoforms in white muscle of the developing turbot (*Scophthalmus maximus*). Basic Appl. Myol. 10(6): 269-278.
- Garfin V X (1990). Ultrastucture and myofibrillar strength of pre-or postrigor CaCl2 injected meat. Proc. 40 th. Inter. congr. of Meat Sci. and Technol. 1-10.
- Habib F S (2010). Taxonomic study of the Clupeidae family types in Iraqi marine waters and the Shatt al-Arab. Master Thesis, College of Agriculture - University of Basra. 150 p.
- Hantosh, Abbas Adel (1998). Seasonal changes in the biochemical content of the muscles of some river and marine fish from the Shatt al-Arab and northwestern Persian Gulf. Master Thesis, College of Science Basra University.
 93 p.
- Hu L, Ren S, ShenQ, ChenJ, Ye X, and Ling J (2017). Proteomic study of the effect of different cooking methods on protein oxidation in fish fillets. RSC Adv., 7: 27496-27505.
- Huda N, Zakaria R, Muchtadi D and Suparno A (1994). Functional Properties of Fish Powder from Selar Kuning (*Selaroides ieptoleptis*). Agric. Biol. Chem. 36:719.
- Kasapis S (2009). Developing mince fish products of improved eating duality: An interplay of instrumental and sensory texture. Int. J. Food Prop. 12:11-26.
- Laemmli U K (1970). Cleavage of structural protein measurement with the Folin phenol reagent .J. Biol. Chem., 193:265-275.
- Liu S, Fan W, Zhong S, Ma C, Li P, Zhou K, Peng Z, and Zhu M (2010). Quality evaluation of tray-packed tilapia fillets stored at 0C based on sensory, microbiological, biochemical and physical attributes. African J. Biotech. 9:692-701.
- Lu H, Zhang L, Shi J, Wang Z and Luo Y (2019). Effects of frozen storage on physicochemical characteristics of bighead carp (*Aristichthys nobilis*) fillets.J. Food processing and preservation. Received: 43 (10):141-151.
- Marcone M R, Kakuda Y, Jahaniaval F, Yada R Y, and Montevirgen L S (2002). Characterization of the proteins of pili nut (*Canarium ovatum*, Engl.). Plant Foods Hum. Nutr.; 57: 107-120.
- Montowska M and Pospiech E (2007). Species identification of meat by electrophoretic methods. ACTA Acta Sci. Pol., Tech. Aliment., 6 (1): 5-16.
- Pineiro C, Barros –Velazquez J, Perez-Martin R I, Martinez I, Jacobsen T, Rehbein H, Kundiger R, Mendes R, Etienne M, Jerome M, Craig A, Mackie I M, and Jessen F (1999). Development of a sodium dodecyle Sulfate– polyacrylamide gel electrophoresis reference method for the analysis and identification of fish species in raw and heat-processed samples. Electrophoresis, 20: 1425-1432.
- Qixing J, ZhengranM, Shuoshuo W, Yanshun X, Fengyu T, Xueqin X, Peipei Y, and Wenshui X (2014). Effect of Temperature on Protein Compositional Changes of Big Head Carp (*Aristichthys nobilis*) Muscle and Exudates. Food Sci. and Techn. Res., 20 (3): 655-661.
- Rodger G, Weddle R B, and Craig P (1980). Effect of time, temperature, raw material type, processing and use of cryoprotective agents on mince quality. In Advances in Fish Sci. and Techn. (J.J. Connell, ed.) :199-217, Fishing News Books, Farnham, Surrey, UK.
- Saeed S, and Howell N K (2002). Effect of lipid oxidation and frozen storage on muscle proteins of Atlantic mackerel (*Scomber scombrus*). J. the Sci. of Food and Agri., 82(5):579-586. https://doi.org/10.1002/jsfa.1080
- Subbaiah K, Majumdar R K, Choudhury J, Priyadarshini B M, Dhar B, Roy D, Saha A, and Maurya P (2015).Protein degradation and instrumental changes in fresh Nile tilapia (*Oreochromis niloticus*) during frozen storage. J. Food Processing and Preservation ISSN 1745-4549. Received for Publication September 22, 2014, Accepted for Publication February 6, 2015:9p.
- Takahashi K, Shirai K, and Wada K (1988). Metting behavior of gels prepared from isolated subunits of collagen. J.Food Sci.53. (6):1365-2621.

www.ejobios.org