Journal of Wasit for Science and Medicine 2026: 19, (3), 29-37

Histochemical Indicators of Muscle Degradation in Three Imported Frozen Fish Products in Al-Kut City

Atyaf Abdul Hasan Hussein[1], and Ahmad Mahdi Saleh[2]

Ministry of Education, Wasit Directorate of Education, Wasit, Iraq.
Department of Biology, College of Sciences, University of Wasit, Wasit, Iraq.

Abstract

Fish products in Iraq are low-priced relative to their high nutritional value, and an important part of the Iraqi people's diet. However, freezing process may affect the nutritional values of these products. The study was conducted to detect nutrient levels, including protein, glycogen, and collagen, in the fish product's meat using histochemical studies. From Iraqi markets, 36 skeletal muscle samples were taken from three types of fish products such as Nile tilapia fillets, Pangasius steaks, and Atlantic salmon portions. All samples were dyed with Bromophenol blue stain, Periodic-acid Schiff stain, Malory`s trichrome stain and measured by the ImageJ program. The histochemical work revealed that muscles of Atlantic salmon portions were the richest in protein, while Pangasius steaks had the lowest protein content. Nile tilapia fillets had the highest glycogen and collagen ratio in this study. Thus, Atlantic salmon portions and Nile Tilapia fillets are good options for dieting.

Keywords: Histochemical, Glycogen, Collagen, and Malory`s trichrome stain.

1. Introduction

Fish meat is a balanced food due to its high nutrient level and affordability, which offers many health advantages [1]. Fish and fish products are good substitutes for red meat, which sometimes causes hazards to human health [2]. Fish muscles have excellence protein, which contains essential amino acids. Moreover, they possess a lower amount of collagen fibers in the connective tissue of fish muscles than red flesh, which improves the digestion of fish flesh [1].

While glycogen in fish muscles is found in lower amounts than in mammals, which lead to the production of less lactic acid upon death [3]. The frozen preservation of fish in long-term causes their widespread distribution, which enhances the condition of the industry's financial status and increases the access of the population to the nutritional advantages of fish [4]. The process of food freezing can reduce the speed of its physical, biological, and chemical deterioration [5].

Freezing causes a prolonging of the shelf life of fish flesh and long-term preservation, but it is known to cause chemical changes, such as denaturation of proteins, which affects the sensory quality of the fish meat [3]. In addition, the tissue structure, proteins, and fatty acids of fish muscle are extremely unstable, and when they are thawed and refrozen, their increased moisture level leads to noteworthy quality alterations [6]. This study was performed to detect the value of nutrients of imported frozen fish products in Iraqi markets by using the histochemical study of the flesh of these fish products.

2. Materials and Methods

Muscle specimens were collected from 36 samples of three types of fish products. Including 12 Nile tilapia (*Oreochromis niloticus*) fillets, 12 Pangasius (*Pangasianodon hypophthalmus*) steaks, and 12 Atlantic salmon (*Salmo Salar*) portions. Muscle samples were removed from the dorsal region of fish products and were kept clean, labeled containers containing 10% formalin for 48 hours.

After that, specimens were subjected to the remaining steps of tissue processing, which were washing with tap water, dehydrating with ethanol gradients, clearing using xylene, infiltration, and embedding in paraffin and cut into 5 µm. Specimens were stained with Bromophenol blue stain (BPB) [7], Periodic-acid Schiff stain (PAS) [8], and Malory`s trichrome stain [9], for detecting protein, glycogen, and collagen content. Whole tissue slides were examined, and photomicrographs were taken. The ratio of surface area of stained section was measured for total protein, glycogen, and collagen using the ImageJ program. The statistical analysis was performed using mean and standard deviation of ratios and the T-test, which was limited to significant differences $p \leq 0.05$.

3. Results and Discussion

3. 1 Histochemical Result

3. 1. 1 Protein Measurements

The histochemical examination showed that the accumulation of protein appeared as a dark blue splatter within the myocytes in the muscle tissue of the studied species. This color was obtained by staining the muscle tissues with BPB stain, as noted in figures 1, 2, and 3. Furthermore, this study revealed that the percentage of total protein in imported frozen fish products was 18.6 ± 1.6%, 14 ± 0.2%, and 20 ± 0.3% in the muscle of Nile tilapia fillet, Pangasius steaks, and Atlantic salmon portions, respectively.

There were significant differences among Pangasius steaks and other fish products (Nile tilapia fillet and Atlantic salmon portions) in the present study, at ($p \leq 0.05$), as listed in table 1. The result of total protein in Nile tilapia fillet coincides with findings of Geremew in Nile tilapia [10]. The ratio of total protein in Pangasius steak, in accordance with Pongpet in frozen P. hypophthalmus [11]. While this result contrasts with the reported value of Chakma who reported the protein percent in farmed P. hypophthalmus was 20.19 ± 0.06% [12].

Also, the protein proportion in Atlantic salmon matched reached in Atlantic salmon [13]. The result demonstrated that the total protein in Atlantic salmon was higher than in other fish products. These results showed that the proportion of total protein in the muscle of the studied species was more than %15. That was considered a high proportion of protein in animal muscles, except that in Pangasius steak, which was below 15% [14]. Through the statistical result of protein ratio, the total protein in Pangasius steak was lower than that of other species in this study. That is probably due to the denaturation of the cellular myofibril proteins during the freezing storage period and proteolysis after postmortem [15].

3. 1. 2 Glycogen Measurements

The microscopically scanning observed the distribution of glycogen in all studied species, which was stained with a dark magenta color by staining with PAS stain. This observation revealed that the scattered distribution of glycogen as small dark magenta-stained granules or spots within myocytes appeared in figures 4, 5, and 6 in the Nile tilapia fillet, Pangasius steaks, and Atlantic salmon portions. Moreover, the proportion of total glycogen in the meat of imported frozen fish products was 0.7 ± 0.09%, 0.6 ± 0.09%, and 0.5 ± 0.03% in Nile Tilapia fillet, Pangasius steaks, and Atlantic salmon portions.

A significant difference in glycogen ratio between Atlantic salmon and Nile tilapia, at probability ($p \leq 0.05$), as listed in table 1. Results of Nile tilapia fillets agreed with in Nile tilapia [16]. The glycogen proportion of Pangasius steak, in line with what was reached in Pangasius pangasius

[17]. The total glycogen percentage in Atlantic salmon concords with the finding of Totland in Atlantic salmon [18]. Moreover, the glycogen in Nile Tilapia was higher than in other species studied. The glycogen level is highly related to animal feeding, these fish are omnivorous, but mainly eat plants, which utilize carbohydrates as energy and have a high ability to accumulate excess carbohydrates as glycogen and lipids [19].

3. 1. 3 Collagen Measurements

Collagen was stained blue by staining the tissue section with Malory`s trichrome stain. The result of the present study observed that the collagen was in endomysium between muscle fibers and in myocommata between myomers, as shown in Figures 7, 8, and 9.

The current study showed that the proportion of total collagen in the muscle of frozen imported fish products was 2.2 ± 0.2%, 1.5 ± 0.4%, and 0.7 ± 0.09% in muscle of Nile tilapia fillet, Pangasius steaks, and Atlantic salmon portions. There were significant differences between types of frozen imported fish products in this study, at (p-value ≤ 0.05) table 1.

The result of total collagen in the muscles of Nile tilapia was close to what found in Nile tilapia [20]. The percentage of Pangasius steaks disagrees with that claimed by Intarak who reported the collagen level in Pangasius bocourti as 3.70% [21]. The ratio of collagen in Atlantic salmon is close to founds in Atlantic salmon [22]. The differences between fish products are due to differences in species and habitat. The collagen is reduced during the freezing process, and the damage to collagen structure is highly related to the postmortem softness, as some researchers reported [23].

Table 1: Measurements of total protein, glycogen, and collagen in Nile tilapia fillet, Pangasius steaks, and Atlantic salmon portions.

Fish products	Protein	Glycogen	Collagen
Nile tilapia fillets	18.6 ± 1.6% A	0.7 ± 0.09% A	2.2 ± 0.2% A
Pangasius steaks	14 ± 0.2% B	0.6 ± 0.09% AB	1.5 ± 0.4% B
Atlantic salmon portions	20 ± 0.3% A	0.5 ± 0.03% B	0.7 ± 0.09% C

*The different capital letters in the same column showed a significant difference within the same column (p-value ≤ 0.05).

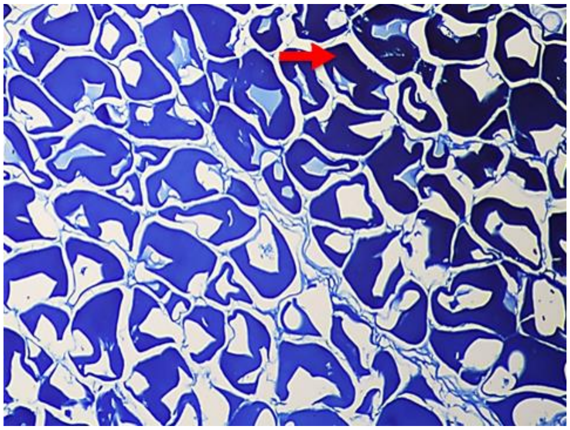

Figure 1: Muscle of Nile tilapia fillet shows the protein content (red arrow). BPB stain, 20 ×.

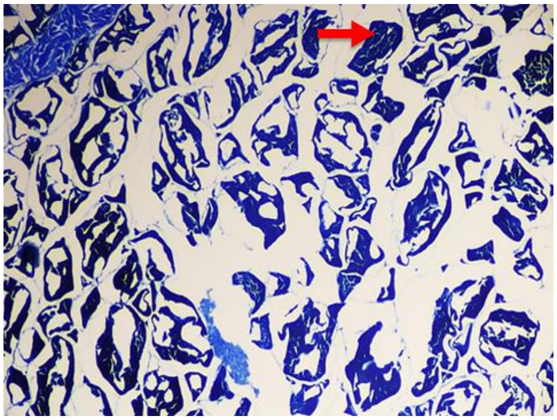

Figure 2: Muscle of Pangasius steak shows the protein content (red arrow). BPB stain, 20 ×.

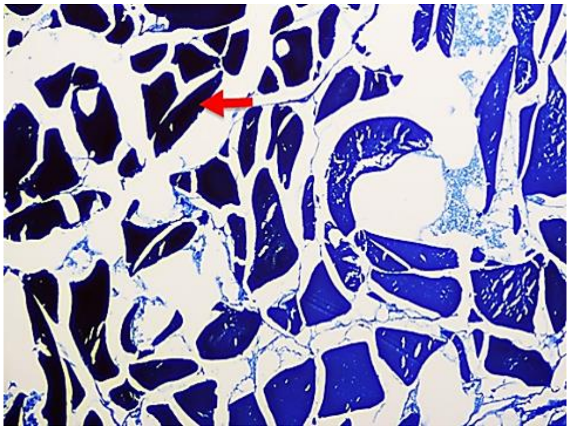

Figure 3: Muscle of Atlantic salmon portion shows the protein content (red arrow). BPB stain, 20 ×.

Figure 4: Muscle of Nile tilapia fillet shows high amount of glycogen content (red arrow). PAS, 40 ×.

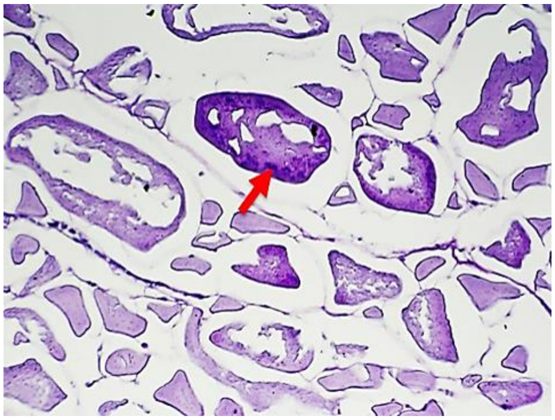

Figure 5: Muscle of Pangasius steak shows the glycogen content (red arrow). PAS stain, 40×.

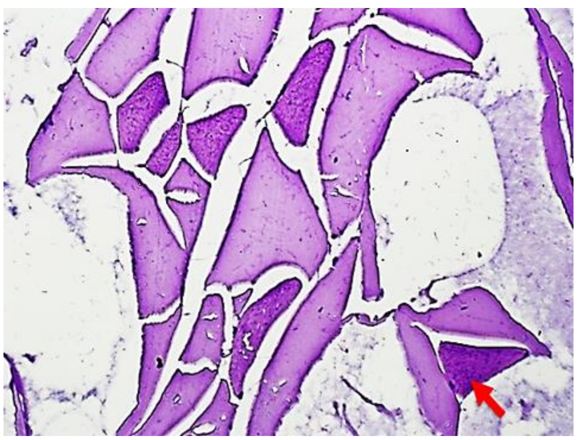

Figure 6: Muscle of Atlantic salmon portion shows the glycogen content (red arrow). PAS stain, 40 ×.

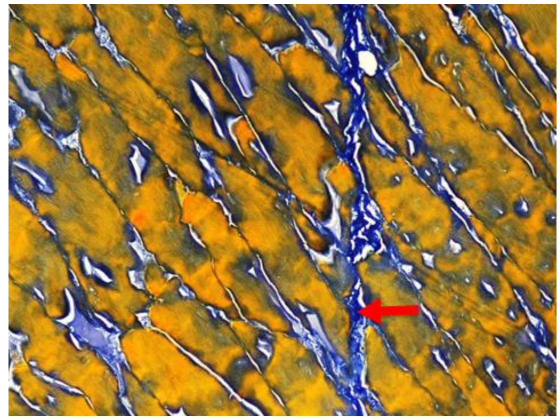

Figure 7: Nile tilapia fillet muscle shows the collagen content (red arrow). Mallory`s Trichrome stain, 20 ×.

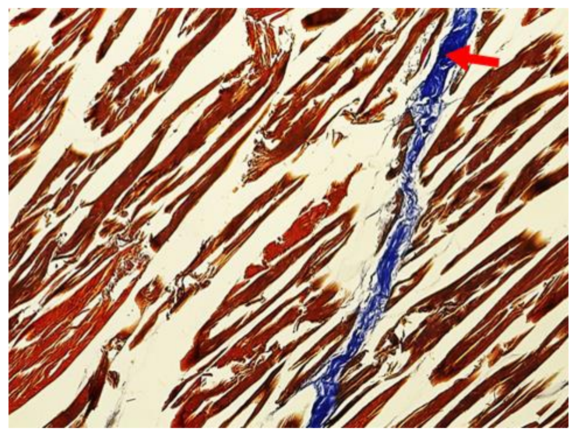

Figure 8: Pangasius steak's muscle shows the collagen content (red arrow). Mallory`s Trichrome stain, 20 ×.

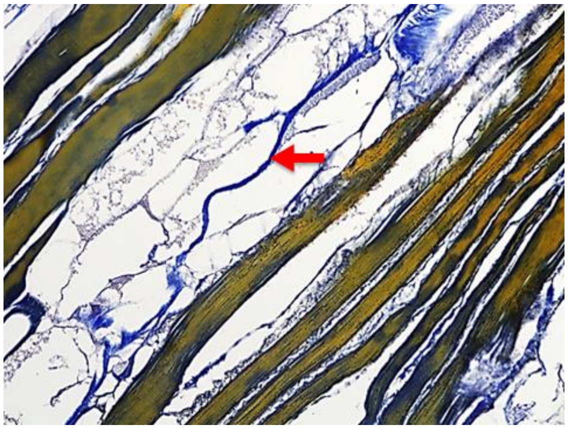

Figure 9: Atlantic salmon portion's muscle shows the collagen content (red arrow). Mallory`s Trichrome stain, 20 ×.

4. Conclusion

Despite the high demand for frozen imported fish products in Iraqi markets due to their low price. Protein content was high in the Atlantic salmon portions and Nile tilapia fillets, but the total protein in the muscles of Pangasius steaks were lowest in this study. This may be caused by deterioration in their tissue structure and denaturation of protein in muscle fibers by effecting of long freezing storage period, poor storage, and temperature fluctuations.

Therefore, Pangasius steaks do not meet the required level of nutrient values of protein. Glycogen content was low in all types of fish products in this study. While collagen content in the muscles of Nile tilapia fillet was greater than that of other types of fish products. This gives hardness to muscle tissue and makes it maintain its tissue texture better than the other types in this study.

5. References

1. Ali A., Wei S., Ali A., Khan I., Sun Q., Xia Q., Wang Z., Han Z., Liu Y., and Liu S., (2022). Research progress on nutritional value, preservation, and processing of fish A review. Foods. 11, 22, 3669.
2. Mahmoud R. A., and Al-Khshali M. S., (2022). Effect of freezing preservation period on some sensory characteristics of three Iraqi local fish species. Iraqi Journal of Agricultural Sciences. 53, 4, 767-773.
3. Gökoğlu N., and Yerlikaya P., (2015). Seafood chilling, refrigeration and freezing: science and technology. 163, 5-37.
4. Al-Jeddawi W., and Dawson P., (2022). The effect of frozen storage on the quality of Atlantic Salmon. Journal of Food Science and Nutrition Research. 5, 2, 552-569.
5. Petzold G., and Aguilera J. M., (2009). Ice morphology: fundamentals and technological applications in foods. Food Biophysics. 4, 4, 378-396.
6. Nakazawa N., and Okazaki E., (2020). Recent research on factors influencing

the quality of frozen seafood. Fisheries Science. 86, 2, 231-244.

7. Chapman D. M., (1975). Dichromatism of bromphenol blue, with an improvement in the mercuric bromphenol blue technic for protein. Stain Technology. 50, 1, 25-30.
8. Suvarna K. S., Layton C., and Bancroft J. D., (2018). Bancroft's theory and practice of histological techniques E-Book. 8th Ed.,75-82. Elsevier Health Sciences.
9. Luna L. G., (1992). Histopathologic methods and color atlas of special stains and tissue artifacts. American Histolabs.
10. Geremew H., Abdisa M., and Goshu G., (2020). Proximate composition of commercially important fish species in southern Gulf of Lake Tana, Ethiopia. Ethiopian Journal of Science and Technology. 13, 1, 53-63.
11. Pongpet J., Ponchunchoovong S., and Payooha K., (2016). Partial replacement of fishmeal by brewer's yeast (Saccharomyces cerevisiae) in the diets of Thai Panga (Pangasianodon hypophthalmus× Pangasius bocourti). Aquaculture nutrition. 22, 3, 575-585.
12. Chakma S., Rahman M. A., Siddik M. A., Hoque M. S., Islam S. M., and Vatsos I. N., (2022). Nutritional profiling of wild (Pangasius pangasius) and farmed (Pangasius hypophthalmus) pangasius catfish with implications to human health. Fishes. 7, 6, 309.
13. Reksten A. M., Ho Q. T., Nøstbakken O. J., Markhus M. W., Kjellevold M., Bøkevoll A., Hannisdal R., Frøyland L., Madsen L., and Dahl L., (2022). Temporal variations in the nutrient content of Norwegian farmed Atlantic salmon (Salmo salar), 2005-2020. Food Chemistry. 373, 131445.
14. Stansby M. E., (1976). Chemical characteristics of fish caught in the northeastern Pacific Ocean. 1-11.
15. Varghese T., and Mathew S., (2017). Assessment of the textural variation of iced stored Anabas testudineus (Bloch, 1792) muscle tissue with emphasis on their collagen and myofibrillar protein content. Journal of Food Science and Technology. 54, 8, 2512-2518.
16. Montoya Camacho N., Márquez Ríos E., Castillo Yanez F. J., Ruíz Cruz S., Arvizu Flores A. A., Torres Arreola W., Ca´rdenas Lo´pez J. L., Valde´z Hurtado S. and Ocaño Higuera V. M., (2020). Changes on the development of rigor mortis in cultured tilapia (Oreochromis niloticus) fed with a mixture of plant proteins. Journal of Chemistry.1, 5934193, 1-9.

17. Rahamat Ullah M., Rahman M. A., Haque M. N., Sharker M. R., Islam M. M., and Alam M. A., (2022). Nutritional profiling of some selected commercially important freshwater and marine water fishes of Bangladesh. Heliyon. 8, 10, e10825.
18. Totland G. K., Kryvi H., Jødestøl K. A., Christiansen E. N., Tangerås A., and Slinde E., (1987). Growth and composition of the swimming muscle of adult Atlantic salmon (Salmo salar L.) during long-term sustained swimming. Aquaculture. 66, 3-4, 299-313.
19. Montoya-Mejía M., Hernández-Llamas A., García-Ulloa M., Nolasco-Soria H., Gutierrez-Dorado R., and Rodríguez-González H., (2016). Apparent digestibility coefficient of chickpea, maize, high-quality protein maize, and beans diets in juvenile and adult Nile tilapia (Oreochromis niloticus). Revista Brasileira de Zootecnia. 45, 08, 427-432.
20. Kayan A., Boontan I., Jaturssitha S., Wicke M., and Kreuzer M., (2015). Effect of slaughter weight on meat quality of Nile Tilapia (Oreochromis niloticus). Agriculture and Agricultural Science Procedia. 5, 159-163.
21. Intarak I., Lhasudta P., Jathurasitha S., Wicke M., and Kreuzer M., (2015). Effects of slaughter weight on carcass and meat characteristics of Punga Fish (Pangasius bocourti Sauvage). Agriculture and Agricultural Science Procedia. 5, 164-169.
22. Bjørnevik M., Espe M., Beattie C., Nortvedt R., and Kiessling A., (2004). Temporal variation in muscle fibre area, gaping, texture, colour and collagen in triploid and diploid Atlantic salmon (Salmo salar L). Journal of the Science of Food and Agriculture. 84, 6, 530-540.
23. Suárez M. D., Abad M., Ruiz-Cara T., Estrada J. D., and García-Gallego M., (2005). Changes in muscle collagen content during postmortem storage of farmed sea bream (Sparus aurata): influence on textural properties. Aquaculture International, 13, 4, 315-325.