

## Evaluation of Physicochemical Properties of Some Imported Frozen Fish Species from Basrah Markets, Iraq

Anfal A. Obeed<sup>1</sup>, Jalal M. Al-Noor<sup>2</sup>

<sup>1</sup>Departement of Fisheries and Marine Resources, College of Agriculture, University of Basrah, Iraq

<sup>2</sup>Unit of Aquaculture, College of Agriculture, University of Basrah, Iraq

\*Corresponding Author: [anfal.ali24@uobasrah.edu.iq](mailto:anfal.ali24@uobasrah.edu.iq)

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### ABSTRACT

The study aimed to evaluate the physicochemical quality parameters of seven species of imported frozen fish in Basrah Governorate, southern Iraq. Fish and fish products can undergo various undesirable changes during frozen storage due to enzymatic and microbial degradation of proteins and fats. Fish samples were collected in three batches at different times: Batch No. 1 in April, Batch No. 2 in July, and Batch No. 3 in September 2024. The physicochemical quality parameters assessed included pH, total volatile nitrogen (TVN), Thiobarbituric Acid (TBA), Free Fatty Acids (FFA), Water Holding Capacity (WHC), drip loss (DL), and myoglobin (Mb). The results showed variations in quality standards among the fish species across the three batches. The pH values ranged from 6.22 to 6.91. TVN levels varied across the batches, ranging from 9.8 to 18.2 mg nitrogen/100 g of fish flesh. TBA values ranged from 0.74 to 1.82 mg malondialdehyde/kg of fish, while FFA values varied from 0.22% to 0.43%. Water holding capacity ranged from 5.66% to 10.11%, and drip loss values ranged from 3.83 to 6.66%. Myoglobin levels showed variations between fish species and batches, with values ranging from 0.043 to 0.154. The findings demonstrated that all fish species were susceptible to significant changes during frozen storage, particularly over extended periods. Although freezing can preserve fish, undesirable changes in fat and protein quality may inevitably occur.

### INTRODUCTION

Fish is one of the food commodities that holds a significant position in global trade and contributes to improving food security (FAO, 2022). It also provides numerous job opportunities in production, fishing, marketing and manufacturing (Issifu & Deffor, 2022). The consumption of fish is no longer limited to its fresh form after being caught; fish processing and its products have become a thriving industry. Many species of fish are frozen and prepared for export to distant markets (Mohamed *et al.*, 2022). Fish are a vital food item with high nutritional value and are considered the second primary source of high-quality animal protein after red meat. They play a crucial role in the diet of nations and countries, distinguished as a highly digestible food that can be prepared in various

ways (Arjunsinh *et al.*, 2024). Fish consumption often depends on the dietary, social, cultural habits and geographical regions of consumers (Pieniak *et al.*, 2011). Furthermore, regular fish consumption offers health benefits, including boosting immunity, protecting against cardiovascular diseases and slowing aging (Samuthirapandian *et al.*, 2010). Today, there is an increasing consumer demand for diverse fish products of the highest quality standards. As a result, the quality and safety of imported fish and fish products are of great concern to food safety authorities, aiming to ensure the sustainable preservation of fish using different preservation techniques (Naylor *et al.*, 2021). Thus, preserving fish and paying attention to its handling and transportation from fishing areas to consumers is essential. Appropriate preservation and storage methods must be followed to maintain the quality and freshness of fish (Sone *et al.*, 2019). Freezing is a widely used method to maintain the high quality of fish and its products, ensuring their suitability for human consumption over relatively long periods. It minimizes undesirable chemical changes, physical damage, and microbial spoilage during storage (Al-Hamdani & Al-Noor, 2024). However, extended freezing periods can lead to various undesirable changes, such as rancidity, bitterness, fishy flavor, and unpleasant taste due to the formation of low-molecular-weight compounds from fat oxidation and protein degradation. Additionally, changes in color, appearance, texture, and water-holding capacity may occur, rendering fish flesh less desirable for some consumers (Malik *et al.*, 2021). Therefore, it is essential to understand the effects of freezing and thawing methods on the textural properties of frozen fish meat and to identify optimal storage conditions that preserve its quality and ensure consumer acceptance (Tocher *et al.*, 2019). Given Iraq's growing trade openness toward the importation of various frozen foods, including fish and its products, to meet local market demands, the current study aimed to estimate the chemical, physical, microbial, and sensory properties of imported frozen fish meat in the markets of Basrah Governorate. The study also focused on evaluating their quality and suitability for human consumption by comparing them with the standard specifications issued by the Central Organization for Standardization and Quality Control. Furthermore, it aimed to classify the fish and identify their scientific and common names to prevent the possible commercial fraud of marketing certain fish species unreliably as premium and expensive varieties.

## MATERIALS AND METHODS

### Raw fish

Seven species of imported frozen fish commonly found in the local markets of Basrah Governorate were used in this study, as shown in Table (1). Fish samples were obtained from a local market in central Basrah specializing in selling frozen fish and food products. The samples were collected in three batches at different dates; Batch No. 1 in April, Batch No. 2 in July, and Batch No. 3 in September 2024. Fish samples were

transported to the laboratory in an insulated polystyrene container cooled with ice. Upon arrival, samples were stored in a freezer at -18°C until further measurements and analyses were conducted.

**Table 1.** List of studied fish

	common name	scientific name
1	Grass Carp	<i>Ctenopharyngodon idella</i>
2	Rohu	<i>Labio rohita</i>
3	Pacu	<i>Piaractus brachypomus</i>
4	Green Mullet	<i>Planiliza subviridis</i>
5	Pompano	<i>Trachinotus</i> spp.
6	Shanak	<i>Acanthopagrus sheim</i>
7	Nuweibi	<i>Otolithes ruber</i>

### **Fish preparation**

The number of fish examined varied depending on the size of the studied fish species, ranging from 5 to 10 fish per species. Each fish species was separated into metal trays and classified by faculty members from the Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah. The length and weight of the fish were measured in both their frozen and thawed states. Fish were then eviscerated by opening them from the ventral side, removing internal organs, fins, scales, skin, and other body components using clean and sharp knives. Fish flesh was isolated, and each part or organ from every fish was weighed separately. The flesh was minced using an electric grinder, and the samples were mixed thoroughly to ensure uniformity. These prepared samples were then used for chemical, quality and sensory analyses.

### **Physicochemical parameter estimation**

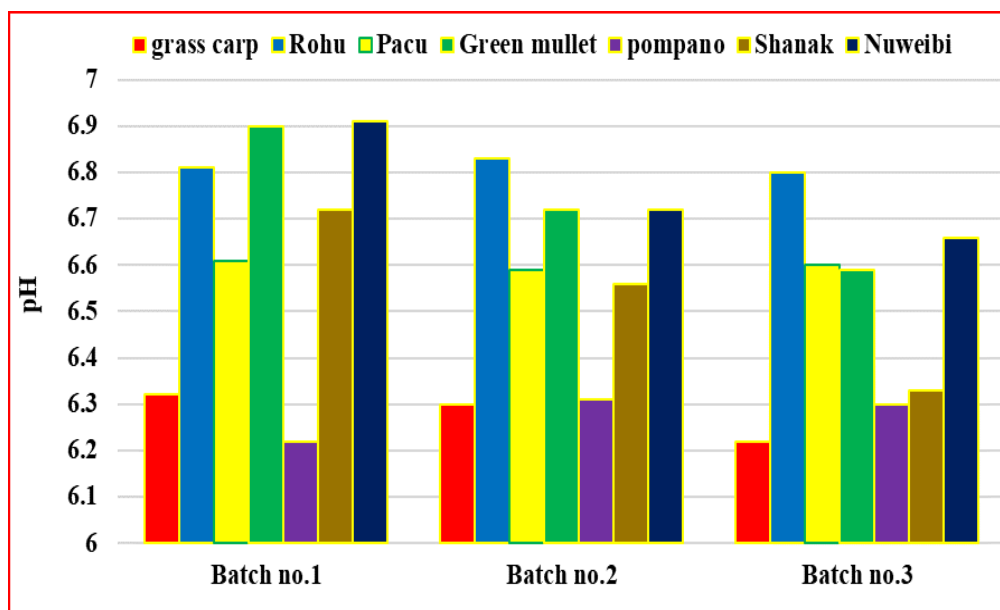
The pH was measured using a pH meter according to the method of **Wong et al. (1991)**. The total volatile nitrogen (TVN) content, expressed as mg nitrogen/100g fish flesh, and the thiobarbituric acid (TBA) value, expressed as mg malondialdehyde/kg fish, were determined following the method of **Egan et al. (1988)**. Free fatty acids (FFA) were measured as oleic acid based on the method of **Wong et al. (1991)**. Water holding capacity (WHC) was estimated using the method detailed by **Stasiak and Dolatowski (1998)**. Drip loss (DL) was determined according to the procedure described by **Young and Lyon (1997)**. Myoglobin concentration was estimated based on the method of **Zessin et al. (1961)**.

## Statistical analysis

The data were statistically analyzed using ANOVA through the SPSS software. Factors were tested based on the LSD (Least Significant Difference) method at a significance level of 0.05, employing a completely randomized design (CRD).

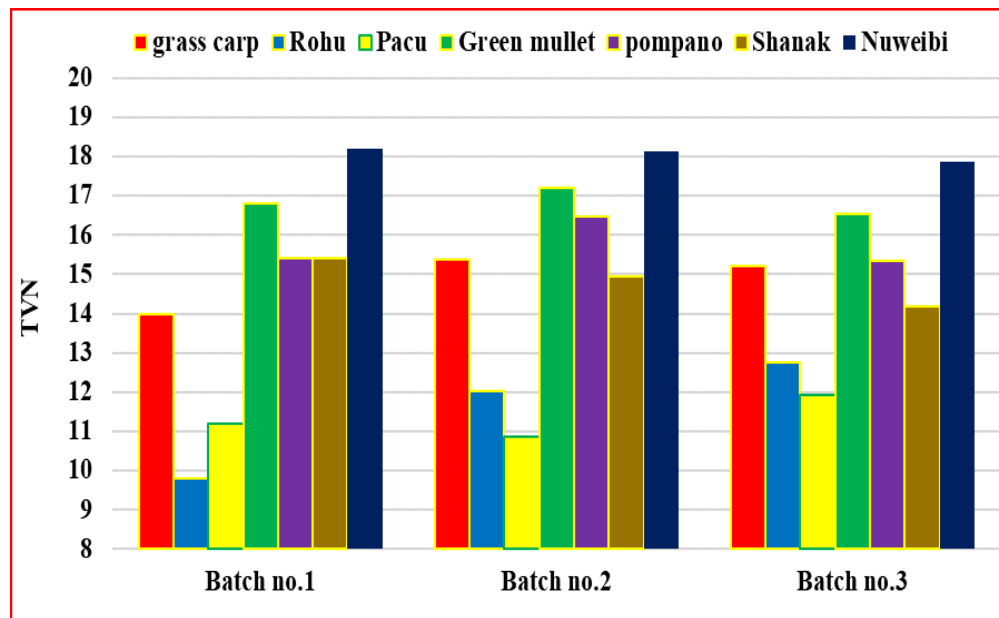
## RESULTS

The results, illustrated in Fig. (1), show pH values of the flesh of the studied fish species across different batches. It is observed that the average pH values of the fish samples oscillate throughout the study batches. The highest pH value was recorded at 6.91 for Nuweibi fish in Batch No.1, with no significant differences ( $P > 0.05$ ) compared to the green mullet and the pompano fish. Conversely, the lowest pH value was 6.22 for the grass carp in Batch No. 3, which was significantly different ( $P < 0.05$ ) from the other fish species across the different batches. The overall mean pH values also varied, with the highest overall mean of 6.81 observed for the rohu fish. No significant differences ( $P > 0.05$ ) were found between the rohu, green mullet, and nuweibi fish. Meanwhile, the lowest overall mean pH value was 6.27 for pompano fish, with no significant differences from the overall mean of the grass carp ( $P > 0.05$ ). The results indicated that the pH values for the grass carp, pacu, green mullet, shanak, and nuweibi were 6.28, 6.6, 6.73, 6.53, and 6.76, respectively. Regarding the effect of the sampling periods on pH values, Fig. (1) shows that the values fluctuated consistently. Overall, the lowest mean pH value was 6.5 in the third batch, while the overall mean increased to 6.57 and 6.64 in the second and first batches, respectively. Significant differences ( $P < 0.05$ ) were observed among the overall means across the examined batches.



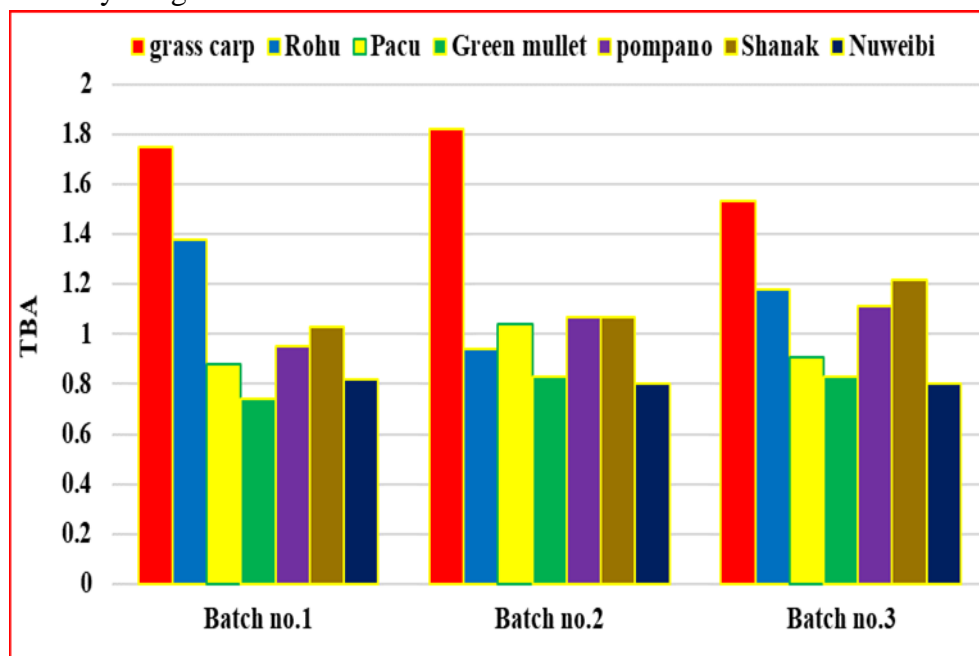
**Fig. 1.** pH values of frozen and imported fish meat across different batches

Fig. (2) illustrates the volatile nitrogenous bases (TVB-N) values of frozen imported fish meat under study. The results indicate that the mean values of TVB-N varied across different batches. Nuweibi fish recorded the highest TVB-N values across all batches, with averages of 18.2, 18.12, and 17.86 for the first, second, and third batches, respectively, showing no significant differences ( $P > 0.05$ ) compared to the rohu fish. On the other hand, the lowest value of TVB-N was observed in Batch No. 19.8 for Rohu fish, which significantly differed ( $P < 0.05$ ) from the grass carp, Ppacu, pompano, and shanak fish. For pacu fish, the lowest TVB-N values were recorded at 10.88 and 11.94 in Batch No. 2 and the third batch, respectively, with significant differences ( $P < 0.05$ ) compared to rohu, pompano, and nuweibi fish. Regarding fish species, the results showed variations in the overall mean TVB-N values, with nuweibi fish achieving the highest overall mean of 18.06. This was not significantly different ( $P > 0.05$ ) from the green mullet fish. In contrast, the lowest overall mean value was recorded for the pacu fish at 11.34, which did not significantly differ ( $P > 0.05$ ) from the rohu fish. The remaining overall means of TVB-N values were 18.87, 11.53, 16.85, 15.74, and 14.84 for the grass carp, rohu, green mullet, pompano, and shanak fish, respectively. Regarding the impact of batch dates on TVB-N values, significant differences ( $P < 0.05$ ) were observed across examined batches. The results indicate that the values fluctuated consistently, with the lowest overall TVB-N mean of 14.4 recorded in Batch No. 1, while the values increased to 15 and 14.83 in the second and third batches, respectively. Statistical analysis confirmed significant differences ( $P < 0.05$ ) in the overall means among various batches.



**Fig. 2.** TVN values of frozen and imported fish meat across different batches

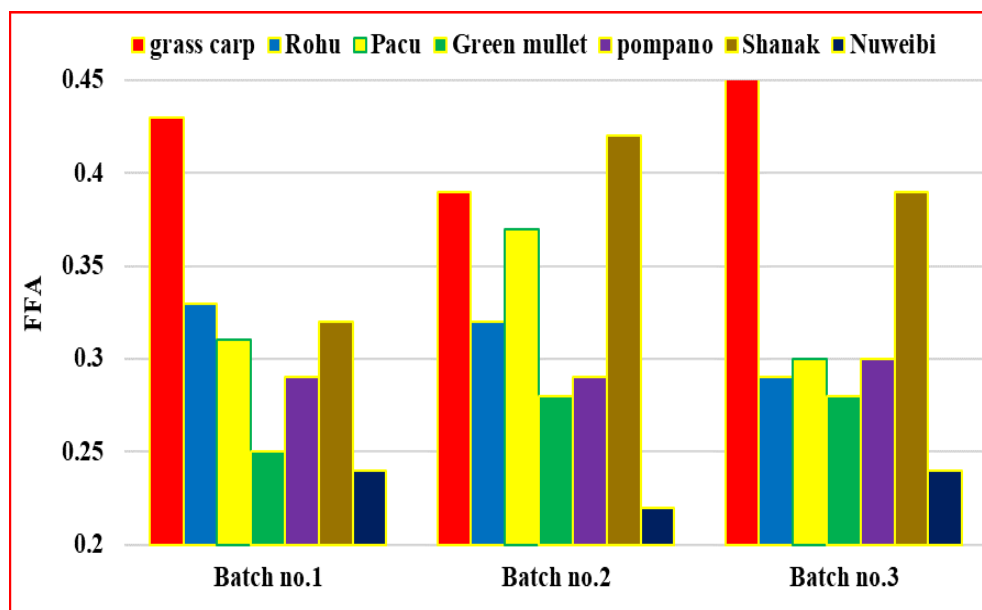
Fig. (3) demonstrates the effect of fish species on the thiobarbituric acid (TBA) value of the studied fish meat. Statistical analysis revealed significant differences ( $P < 0.05$ ) in TBA values among fish types. The grass carp recorded the highest TBA values in the first, second, and third batches, with averages of 1.75, 1.82, and 1.53mg malondialdehyde/kg fish, respectively. In contrast, the lowest TBA values were observed in the green mullet, with 0.74mg malondialdehyde/kg fish in Batch No. 1, and in the nuweibi fish, with 0.80mg malondialdehyde/kg fish in both the second and third batches. Statistical analysis revealed significant differences ( $P < 0.05$ ) among fish types across the different batches. Regarding the overall means, statistical analysis indicated significant differences ( $P < 0.05$ ) in the general averages of TBA values across fish types. The grass carp recorded the highest overall mean value of 1.7mg malondialdehyde/kg fish. Conversely, the lowest overall means were recorded for the green mullet and nuweibi fish, with averages of 0.8mg malondialdehyde/kg fish, showing no significant differences ( $P > 0.05$ ) compared to the overall mean for the pacu fish. The overall means for rohu, pacu, pompano, and shanak fish were 1.16, 0.95, 1.04, and 1.1mg malondialdehyde/kg fish, respectively, with significant differences ( $P < 0.05$ ) observed among the remaining fish species. The TBA value was significantly affected ( $P < 0.05$ ) by batch dates, with closely similar values of 1.07mg malondialdehyde/kg fish in the first Batch and 1.08mg malondialdehyde/kg fish in both the second and third batches.



**Fig. 3.** TBA values of frozen and imported fish meat across different batches

Fig. (4) presents the free fatty acid (FFA) values of frozen imported fish meat under study. The results show that the mean FFA values varied across different batches. The grass carp exhibited the highest FFA values, averaging 0.43 in Batch No. 1 and 0.47 in Batch No. 3, with significant differences ( $P < 0.05$ ) compared to other fish species

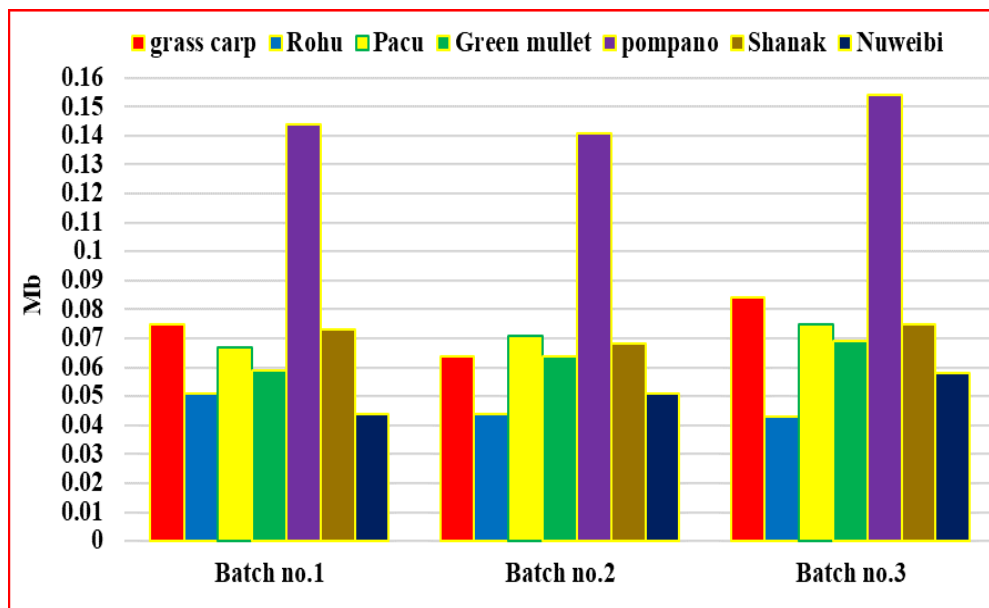
during these batches. Shanak fish recorded the highest FFA value in Batch No. 2, with an average of 0.42. Conversely, the lowest FFA values were observed in nuweibi fish, with averages of 0.24, 0.22, and 0.24 for Batches No. 1, 2, and 3, respectively, showing significant differences ( $P < 0.05$ ) from other fish species across all batches. Regarding fish species, the results demonstrated variations in the overall mean FFA values. The grass carp recorded the highest overall mean of 0.43, with no significant differences ( $P > 0.05$ ) compared to the overall mean for shanak fish across all batches. The lowest overall mean was observed in nuweibi fish, at 0.23, with no significant differences ( $P > 0.05$ ) compared to the overall mean for Green mullet. The remaining overall FFA values were 0.31, 0.32, 0.27, 0.29, and 0.37 for the rohu, pacu, green mullet, pompano, and shanak fish, respectively. As for the effect of batch dates on FFA values, Fig. (4) shows that values fluctuated over time. Overall, the lowest mean FFA value of 0.31 was recorded in Batch No. 1, while the values increased slightly to 0.32 in batches No. 2 and 3. Statistical analysis indicated significant differences ( $P < 0.05$ ) in the overall mean FFA values among batches.



**Fig. 4.** FFA values of frozen and imported fish meat across different batches

Fig. (5) illustrates the myoglobin values of the studied fish meat types across different batches. The results indicate that the mean myoglobin values for fish samples fluctuated between increases and decreases across the batches. Pompano fish recorded the highest myoglobin values in all batches, with averages of 0.144, 0.141, and 0.157 for the first, second, and third batches, respectively, showing significant differences ( $P < 0.05$ ) compared to other fish species across all batches. Conversely, the lowest myoglobin value was recorded for nuweibi fish in Batch No. 1, at 0.044, compared to the lowest values of 0.044 and 0.043 for Rohu fish in the second and third batches, respectively. Statistical analysis revealed significant differences ( $P < 0.05$ ) among the remaining fish

species across the batches. Regarding fish type, the overall mean myoglobin values varied significantly. Pompano fish recorded the highest overall mean value of 0.146, while rohu fish had the lowest, with an overall mean of 0.046. The overall means for the remaining fish species were 0.074, 0.071, 0.064, 0.072, and 0.051 for the grass carp, pacu, green mullet, shanak, and nuweibi fish, respectively, with significant differences among the overall means of fish species across the batches. As for the effect of batch dates on myoglobin values, there was a noticeable variation in values across the batches. The highest overall mean value was recorded in Batch No. 3, at 0.079, followed by Batch No. 1, with an overall mean of 0.073. The overall mean for Batch No. 2 was 0.071. Statistical analysis indicated significant differences ( $P < 0.05$ ) in the overall mean myoglobin values across the batches.



**Fig. 5.** Mb values of frozen and imported fish meat across different batches

Fig. (6) highlights the effect of fish species on the water-holding capacity (WHC) of the studied fish meat. Statistical analysis revealed significant differences ( $P < 0.05$ ) in WHC values among fish types across the batches. Pompano fish recorded the highest WHC values in the first, second, and third batches, with averages of 10.11, 10.03, and 9.97, respectively, showing no significant differences ( $P > 0.05$ ) compared to pacu and the green mullet fish. Conversely, the lowest WHC values were observed in the grass carp, with averages of 5.82, 5.72, and 5.66 for the first, second, and third batches, respectively, with no significant differences ( $P > 0.05$ ) from the remaining fish species across the batches. Regarding the overall means, statistical analysis indicated significant differences ( $P < 0.05$ ) in the general averages of WHC values among fish species. Pompano fish recorded the highest overall mean WHC value of 10.03, while the grass carp had the lowest mean of 5.73, showing a significant difference ( $P < 0.05$ ) compared to the overall means of the other fish species. The overall WHC means for rohu, pacu, the green mullet,



shanak, and nuweibi fish were 6.99, 9.36, 7.33, 6.87, and 8.92, respectively. The WHC values were significantly influenced ( $P < 0.05$ ) by the batch date, with fluctuating values across the different batches. The overall mean WHC values were 7.98, 7.87, and 7.82 for the first, second, and third batches, respectively. Statistical analysis confirmed significant differences ( $P < 0.05$ ) in the WHC values across the batches.

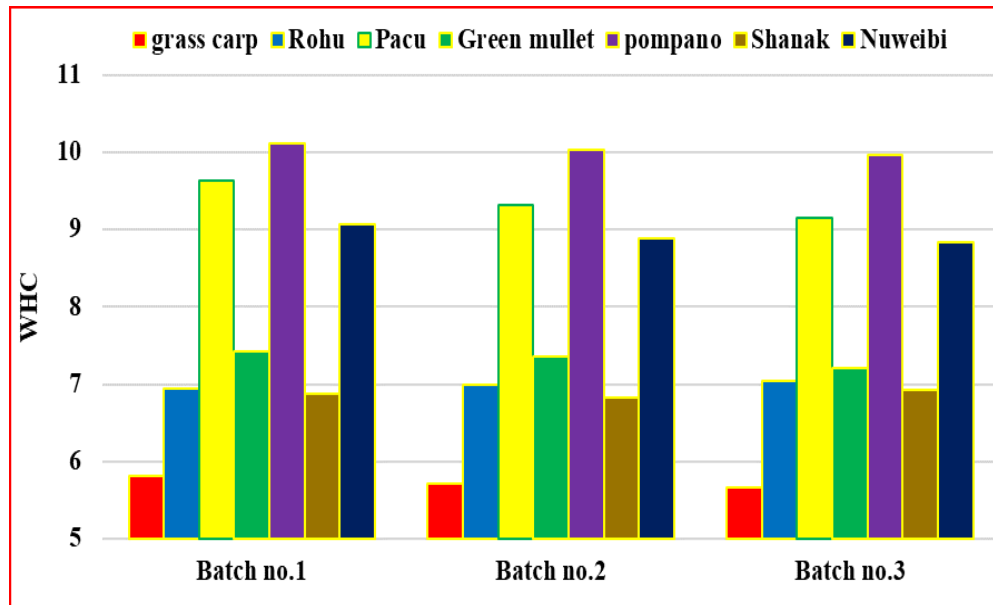


Fig. 6. WHC values of frozen and imported fish meat across different batches

Fig. (7) presents the thawing loss values for the frozen imported fish meat under study. The results indicate that the mean thawing loss values varied across the batches. Shanak fish exhibited the highest thawing loss values across all batches, with averages of 6.55, 6.65, and 6.66 for the first, second, and third batches, respectively, showing significant differences ( $P < 0.05$ ) compared to other fish types across the batches. Conversely, the lowest thawing loss values were observed in pompano fish in Batch No. 1, with an average of 3.83, showing significant differences ( $P < 0.05$ ) from the other fish species across the batches. Similarly, pacu fish recorded lower thawing loss values at 3.97 and 4.16 in the second and third batches, respectively, with no significant differences ( $P > 0.05$ ) compared to Rohu fish. Regarding fish species, the results demonstrated significant variations in the overall mean thawing loss values. Shanak fish recorded the highest overall mean of 6.62, showing significant differences ( $P < 0.05$ ) compared to the overall means of other fish types across the batches. The lowest overall means were observed for pacu and pompano fish, at 4.00, with no significant differences ( $P > 0.05$ ) compared to the overall means of other fish species. The overall thawing loss means for the grass carp, rohu, green mullet, and nuweibi fish were 5.69, 6.25, 5.75, and 5.28, respectively. For the effect of batch date on thawing loss values, significant differences ( $P < 0.05$ ) were observed among the overall means across the batches. The lowest overall mean thawing loss value was recorded in Batch No. 1 at 5.25, while the

values increased to 5.35 and 5.51 in the second and third batches, respectively. The results demonstrate a continuous variation in thawing loss values across the batches.

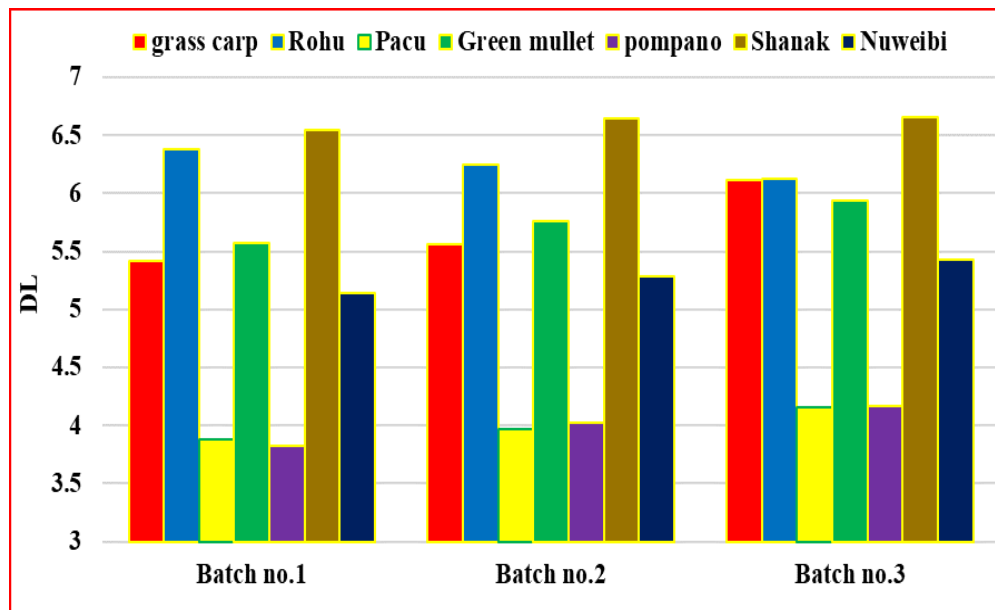


Fig. 7. DL values of frozen and imported fish meat across different batches

## DISCUSSION

The results revealed variability in pH values among the studied fish species and across the batches. This variability is attributed to differences in the levels of carbohydrate compounds (glycogen) in the flesh of each fish species, depending on their chemical composition. These differences lead to variations in the amount of lactic acid formed postmortem, which is responsible for changes in pH values and the biochemical changes that occur after the fish's death, such as protein degradation (Valizadeh *et al.*, 2020). The pH value can serve as an indicator of fish spoilage and an assessment of meat quality (Cheng *et al.*, 2022). The increase in pH is caused by the production of volatile basic compounds, such as ammonia and trimethylamine, by endogenous enzymes or microorganisms in the fish meat during frozen storage. This leads to an elevation in pH values (Bu *et al.*, 2022). The observed variability in pH values in frozen fish agrees with findings from previous researches. For instance, Badee *et al.* (2013) reported an increase in average pH values in frozen fish samples due to the formation of nitrogenous compounds resulting from enzymatic and microbial protein degradation. Additionally, pH levels can be influenced by the formation and release of inorganic phosphates and nitrogenous compounds, which vary depending on fish species and chemical composition (Sun *et al.*, 2019). Diao *et al.* (2021) stated that cell damage, reduced water retention, water loss, and the release of hydrogen ions primarily contribute to a decrease in pH. Similarly, Tan *et al.* (2019) found that freezing with glazing effectively lowered pH values and maintained water-holding capacity in squid stored frozen for six months

compared to control samples. **Liu et al. (2023)** observed in their study on three species of freshwater fish that initial pH values ranged from 6.5 to 7, decreasing to 6.29 after two weeks of frozen storage. **Obemeata et al. (2011)** also noted that variations in pH values among frozen fish samples were due to protein denaturation and an increase in water-soluble compounds caused by drip loss. Changes in the pH of fish muscles during different freezing durations may vary depending on fish species and other biological factors. In general, fish meat is considered acceptable if the pH value reaches 6.8. However, when it exceeds 7, it is deemed spoiled (**Huss, 1995**).

The results indicated clear differences in volatile nitrogen base (TVN) values among the studied frozen fish samples across the different batches. The increase in total volatile nitrogen (TVN) content in frozen fish is attributed to protein hydrolysis by protease enzymes secreted by proteolytic bacteria and the accumulation of alkaline compounds such as ammonia and trimethylamine resulting from protein breakdown (**Duarte et al., 2020**). Additionally, the temperature during the study period and the timing of sample collection from markets played a role in the variability of TVN values, particularly due to the thawing process, which promotes microbial growth. These microbes secrete various enzymes, including proteolytic enzymes, leading to protein degradation and the formation of low-molecular-weight compounds such as peptides and peptones, which gradually increase TVN values (**Karami et al., 2013**). The findings align with those of **Bhujbal et al. (2021)**, who observed an increase and variability in TVN values in the Nile tilapia (*Oreochromis niloticus*) fillets. Similarly, **Nazemroaya et al. (2011)** reported an increase of 15mg nitrogen/100g fish after six months of storage at -18°C, attributing the rise to the breakdown of nitrogen-containing compounds into various amines. **Mohmaudzadeh et al. (2010)** found a significant increase in TVN values in flatfish (*Pseudorhombus elvatus*) and lizardfish (*Saurida undosquamis*) stored at -18°C for five months, with a marked elevation observed at the end of the second month of storage. They attributed the elevated TVN levels to bacterial spoilage and endogenous enzymatic activity. **Seifzadeh et al. (2012)** studied TVN values in frozen *Clupeonella cultriventris* and found levels of 21.7mg nitrogen/100g fish. **Ninan et al. (2008)** reported TVN values ranging from 12.4 to 20.2mg nitrogen/100g fish for tilapia (*Oreochromis mossambicus*). **Wang et al. (2022)** noted that total volatile nitrogen values in fish are considered acceptable if they are below 20mg nitrogen/100g fish, whereas values exceeding this threshold indicate the loss of freshness. The current study's findings demonstrated that the TVN values for the studied species were within the permissible limits.

The results indicate that thiobarbituric acid (TBA) values were influenced by the species of frozen fish and the studied sample batches. TBA levels depend on various factors, including fishing and handling methods, the duration and method of processing, freezing time, and the production and expiration dates (**Fan et al., 2009**). The increase in

TBA values can be attributed to lipid autoxidation, leading to the production of aldehydes and ketones during frozen storage (**Tingting et al., 2012**). In some cases, a slight decrease in TBA values in frozen and fresh fish samples may occur due to the slower lipid oxidation process. The degree of lipid oxidation can be reflected by secondary oxidation products, which are assessed based on TBA values (**Hac-Szymanczuk et al., 2019**). Repeated freezing and thawing of fish can lead to a rapid increase in TBA values (**Cheng et al., 2019**). The findings are consistent with **Karami et al. (2013)**, who observed an increase in TBA values in the red tilapia (*Tilapia mosambicus*) and the Nile tilapia (*Oreochromis niloticus*) fillets during frozen storage, indicating lipid oxidation during this period. Similarly, **Liu et al. (2010)** reported elevated TBA levels in frozen fish, highlighting the variability of this reaction between fish species due to differing oxidation mechanisms during storage. **Jeong et al. (2021)** also observed an increase in TBA values in certain freshwater fish during frozen storage, attributing these changes to the high content of polyunsaturated fatty acids (PUFAs) in freshwater fish, which are prone to oxidation, leading to secondary oxidation products like peroxides and malondialdehyde. **Karami et al. (2013)** recorded significant increases in TBA values during frozen storage due to the oxidation of unsaturated fatty acids, which contribute to lipid degradation over time. **Liu et al. (2010)** observed that the rise in TBA levels in frozen fish meat is caused by lipid oxidation and the reaction of final oxidation products, such as malondialdehyde and aldehydes, with other compounds in the fish, including amines, nucleotides, nucleic acids, and proteins. These reactions vary significantly among fish species, leading to variations in TBA values during frozen storage. The acceptable limit for TBA in fish meat is 2mg malondialdehyde/kg fish (**Huss, 1995**). In the present study, the TBA values for the samples were within acceptable limits.

The levels of free fatty acids (FFAs) varied among fish species within the studied batches. This variation might be attributed to the activity of lipase enzymes, which break ester bonds and release FFAs. These acids are the end products of lipid hydrolysis caused by lipases and phospholipases produced by fat-degrading bacteria (**Fadılođlu & Coban, 2018**). Consequently, the production of FFAs serves as an indicator of the progression of lipid oxidation or hydrolysis and can be used as a metric to determine the degree of lipid deterioration and the quality of food products (**Mehrabi et al., 2021**). The findings of the current study align with those of **Khidhir et al. (2013)**, who studied frozen fillets of tuna, salmon, and mackerel. Similarly, **Pawed et al. (2013)** reported an increase in FFA values in freshwater fish *Catla catla* during their evaluation of biochemical and sensory characteristics under frozen storage at -18°C. The differences in fatty acid composition play a crucial role in forming hydroperoxides, as oxidative changes in the fats of frozen fish are often driven by the presence of free radicals, which are markers of this process during frozen storage. The FFA values in the fish samples of the current study were within acceptable limits, with **Huss (1995)** suggesting that acceptable FFA limits range between 1–2.

The results also indicated variations in myoglobin concentrations among the frozen fish species and the examined sample batches. Changes in color and appearance, such as loss of tissue color intensity, surface gloss, water loss, reduced transparency, and a chalky appearance in muscle tissues, result from imbalanced changes in muscle tissue proteins or pigment proteins like myoglobin and oxymyoglobin, which degrade into metmyoglobin. This process can be influenced by several factors, including pH, temperature, oxygen consumption and reaction, diet quality, surrounding environment, physiological state of the fish and gender (**Aguilar *et al.*, 2000**). Additionally, variations in myoglobin pigment can be attributed to reduced protein content, which correlates positively with red muscles that are rich in capillaries supplying hemoglobin and providing energy during swimming (**Zhao *et al.*, 2014**). **Zhan *et al.* (2018)** highlighted that changes in myoglobin pigmentation are closely associated with lipid oxidation and the formation of secondary oxidation products, such as peroxides, which vary among fish species and negatively affect color (**Li *et al.*, 2022**). Furthermore, the decline in myoglobin concentration in fish meat can be attributed to protein denaturation and oxidation, as well as freezing and thawing durations. These factors contribute to the loss of muscle color in fish. These results are consistent with **Li *et al.* (2023)**, who noted that fish myoglobin is more prone to oxidation than mammalian myoglobin, leading to a color change from bright red to dark brown, a decrease in the red color index, and an increase in yellow pigmentation. **Lin *et al.* (2021)** studied the effect of muscle protein oxidation in white shrimp and found that higher hydroxyl radical concentrations negatively affected color by actively attacking amino acids and protein structures, which reduced protein stability. These findings align with **Amine *et al.* (2023)**, who monitored protein oxidation and its effect on myoglobin oxidation in frozen fish and fishery products.

Changes in texture during frozen storage are directly related to protein denaturation and the sensory properties of fish muscles (**Lorentzen *et al.*, 2020**). Water-holding capacity (WHC) is often associated with post-mortem structural changes, including the degradation of the muscle matrix and myosin protein denaturation (**Dawson *et al.*, 2018**; **Xie *et al.*, 2022**). The decline in WHC during frozen storage may be attributed to moisture loss from the meat's surface. Several pre- and post-harvest factors, such as stress, feeding conditions, and *rigor mortis*, can influence WHC values (**Rotabakk *et al.*, 2018**). **Zhang *et al.* (2019)** reported a decrease in WHC values due to the growth of ice crystals during frozen storage, which significantly contributes to protein denaturation. Changes in WHC in frozen fish muscles after thawing are clearly affected by freezing storage and are linked to alterations in myofibrillar proteins (**Tan *et al.*, 2018**). Variations in weight loss between treatments during frozen storage can be attributed to water sublimation. Glazing acts as a protective barrier against water vapor, thereby reducing moisture loss from samples. Consequently, water loss decreases gradually, making its removal more difficult and resulting in a gradual increase in WHC during the later stages of frozen storage. Additionally, glazing prevents mechanical

damage to muscle structure, preserves proteins from denaturation, and minimizes negative effects during thawing (Wang *et al.*, 2020). Wang *et al.* (2022) confirmed a decline in the water retention capacity of frozen tuna (*Thunnus thynnus*) stored at -18°C for 180 days. Taliadorou *et al.* (2003) observed WHC percentages of 21 and 19% for frozen sea bass and sea bream, respectively. Zhu *et al.* (2019) found that rapid freezing minimizes the formation of large ice crystals, thereby reducing damage to muscle fibers, particularly myofibrillar proteins. This explains the variation in WHC among fish species depending on the degree of damage to muscle tissue (Sun *et al.*, 2019). The current study's results are consistent with previous studies that reported variations in WHC percentages among fish species during frozen storage due to decreased intercellular binding (Dalvi-Isfahan *et al.*, 2019; Luo *et al.*, 2020; Wei *et al.*, 2021).

The results also indicated that differences in thawing loss percentages could be due to free water. This type of water has weak binding and is primarily found on the surface of meat, making it easily lost even during simple processing operations. If fish meat loses a larger amount of free water, the drip loss will also be higher (Park *et al.*, 2021). The higher thawing loss percentage may result from structural damage caused by large ice crystals. Additionally, this could be due to the melting of ice crystals formed within cells or around muscle tissues during frozen storage. Protein denaturation during storage is primarily responsible for changes in fish quality, including stiffness, sponginess, reduced tenderness, dryness, elasticity, and loss of water-holding capacity (Naseri *et al.*, 2020). Ma *et al.* (2021) noted that the formation and growth of ice crystals during freezing could lead to water migration from inside the cells to the outside, along with varying degrees of cellular water loss and solute concentration increases. Bhujbal *et al.* (2021) observed an increase in water loss across all treatments, ranging from 2.18% on the first day to 4.82% after 120 days of storage, and reported that water loss varies depending on the treatment. Similar results were observed by Pawar *et al.* (2013) in frozen fish catla (*Catla catla*).

## CONCLUSION

In conclusion, from the results of this study, fish contained a good composition and proportions of high-nutritional-value amino acids and fatty acids, unaffected by the freezing preservation method. The chemical and physical quality attributes of the fish remained within the recommended limits. Overall, pompano, pacu and otolithes fish scored higher in most quality attributes.

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