LIPID OXIDATION CHANGES IN TERMS OF PV AND TBA IN WHOLE *PAMPUS ARGENTEUS* FROM VISAKHAPATNAM STORED AT -20°C

Dr. Y. Shanti Prabha[*1] and Prof. C. Manjulatha[2]

[1]Senior Lecturer in Zoology, Dr. V.S. Krishna Government Degree and PG College (Autonomous), Visakhapatnam-530 013

[2]Department of Zoology, College of Science and Technology, Andhra University, Visakhapatnam-530 003

*Corresponding Author Email: shantiprabhay@gmail.com

Article History: Received: 08-05-2023 **Revised:** 18-06-2023 **Accepted:** 29-06-2023

Abstract

Lipid oxidation, especially in marine products with high lipid concentrations, is a significant factor in the quality of the product deterioration. The large concentration of highly polyunsaturated fatty acids found in species makes fish particularly susceptible to this fundamental deteriorative process, even though it occurs in many different types of animal sources of food. The aim of the present study was to determine the changes in Peroxide Value and Thiobarbituric Acid Value (TBA) as chemical quality indices of lipid oxidation in whole *Pampus argenteus* from Visakhapatnam harbor during frozen storage at -20°C. Results obtained conclude that the temperature of storage should be lower than -20°C so as to maintain the limits of acceptability for a longer duration.

Keywords: *Pampus*, PV, TBA.

Introduction

Polyunsaturated fatty acids (PUFA), particularly eicosapentaenoic (EPA, 20: 5ù-3) and docosahexaenoic acids (DHA, 22: 6-3), are abundant in marine lipids (Pazos *et al.,* 2005; Bayir *et al.,* 2006). Consuming fish high in polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has been proven to have both therapeutic and preventive effects on cardiovascular disease, cancer, hypertension, inflammation, fat glycemic management, and baby neurodevelopment (Von Schacky, 2000; Kris-Etherton *et al.,* 2003;Sidhu, 2003).The demand for high-quality seafood is growing every year due to factors including population growth and a preference of higher protein diet intake brought on by an ageing population (Cai and Leung, 2017). The sustainability of our ecosystems, however, makes it difficult to increase both wild fisheries and aquaculture (Alexandratos and Bruinsma, 2012).

Fish quality changes do occur during chilling and frozen storage. Although traditional methods like freezing and chilling are still the most often used procedures onboard, it is still not obvious how frozen fish degrades. This needs to be understood for products that need to be kept frozen for a long time and maintain high quality. Fish can be stored refrigerated (between 0 and 4 degrees Celsius) or frozen (between -18 and -40 degrees Celsius) in order to lengthen their shelf life and maintain their freshness at low temperatures (Walayat *et al.,* 2020). One of the most crucial methods for the long-term preservation of fish muscle is

frozen storage. However, physicochemical and structural changes continue to occur (Rodríguez Herrera *et al.*, 2000). Fish tissues are extremely vulnerable to oxidation and quick degradation due to the high proportion of unsaturated lipids in their tissues. Fish flavour and texture are mainly affected by oxidative changes. Changes in colour and nutritional value are seen during later stages of lipid peroxidation (Dragoev *et al.*, 1998). Fish oils and fatty fish easily produce volatiles linked to rancidity due to PUFA degradation by auto-oxidation during storage and processing (Pazos *et al.*,2005). Fish that has been frozen can help prevent microbial deterioration, however lipid oxidation may also be a contributing spoilage factor (Badii and Howell, 2002).

Lipid oxidation is a very complicated and significant process that jeopardises the quality of food, particularly that which has a large proportion of unsaturated fats. The majority of polyunsaturated fatty acids come from fish, which is problematic because they are easily degraded by processes like oxidation. In fact, lipid oxidation has a significant role in the quality of foods, particularly those high in highly unsaturated fats. Some of the several effects of lipid oxidation in foods include quality losses, development of disagreeable flavour and aroma, shortening of shelf life, nutritional value losses (such as loss of polyunsaturated fatty acids, PUFAs), and potential production of harmful compounds. Since it causes quality to decline and reduces the marketability of fish products, lipid oxidation is a key concern during the processing and storage of fish (Secci and Parisi, 2016). The development of secondary lipid oxidation products, such as the oxidation of peroxides into aldehydes and ketones, is indicated by thiobarbituric acid or thiobarbituric acid reactive substances (TBARS), which are widely employed to measure the degree of fish oxidation. The secondary malondialdehyde (MDA) compound extraction serves as the basis for the calculation of the TBA index (Hocaoğlu *et al.*, 2012).

The present study has been taken up to study the changes in Peroxide values and TBA chosen as the chemical quality indices of lipid oxidation in whole *Pampus argenteus* collected from Visakhapatnam harbor and stored at -20°C for a duration of 180 days.

Materials and Methods

The present study includes studies on Peroxide value and TBA measurements of one of the most preferred marine species namely *Pampus argenteus* landed at Visakhapatnam fishing harbor. All the fresh samples were collected from Visakhapatnam Fishing harbour. Without any time lapse the tests were undertaken after thorough washing of the fish with water in the lab. The samples of fish were not eviscerated as the present study was on whole fishes. The sample was separated in 2 lots. The first lot was analysed in fresh condition and the second lot was packed in sterile polythene bags in the whole form and was stored at -20°C. The frozen samples were analysed across fifteen durations of storage i.e., after 1, 3,5,7,14,21, 28, 42, 56, 70, 84, 120, 150, 180 days of storage.

Determination of Peroxide (PV)

Peroxide value was determined by according to Egan *et al.*, 1981 in Pearsons's chemical analysis of foods. Minced muscle was blended with twice its weight of anhydrous sodium sulpahte in mortar. The blend was shaken with distilled chloroform for 5 to 10 minutes and filtered. For PV estimation 5 gms of oil was taken into 250 ml boiling conical flask., 30 ml of HOAc–$CHCL_3$ was added and swirled to dissolve, 0.5 ml saturated KI solution was added, shaken thoroughly and was boiled in waterbath for not more than 30 seconds. 30 ml of water was addedslowly and then the liberated iodine was titrated with 0.1

N $Na_2S_2O_3$ with vigorous shaking until yellow was almost gone. 0.5 ml of 1% Starch solution was added and titrated by shaking vigorously so as to release all the iodine from the chloroform layer until blue colour just disappeared. Blank determination was carried out simultaneously. The Peroxide Value is often reported as the number of ml of 0.002 N Sodium thiosulphate per gram of sample. The value so obtained was multiplied by 2, which then equals milliequivalents of peroxide oxygen per kg of sample (meq/kg).

Determination of Thiobarbituric Acid Value (TBA Value)

Thiobarbituric acid value was estimated according to the method described by Vynke, 1970. 10 gms of fish muscle was homogenized with 50 ml distilled water and washed into distillation flask with 47.5 ml distilled water. 2.5 ml 4N HCL was added and heated by adding glass beads. 50 ml of distillate was collected in 10 minutes. 5 ml distillate was pipette into a glass stoppered tube; 5ml TBA reagent was added, stoppered, and heated in boiling water bath for 35 minutes. A blank was similarly prepared using 5ml distilled water with 5ml reagent, then the tubes were cooled and OD was measured against the blank at 538 nm. TBA number as mg Malonaldehyde per kg sample is equal to O.D. x 7.8.

Results

Initial Peroxide value in fresh *Pampus argenteus* was 0.17 meq/kg and it decreased to 0.15 meq/kg after one day of storage. After 3 days PV increased to 0.33 meq/kg. With a continuous increase PV reached 16.42 meq/kg after 84 days of frozen storage. On further storage PV decreased and reached 11.62 meq/kg at the end of 180 days of frozen storage.

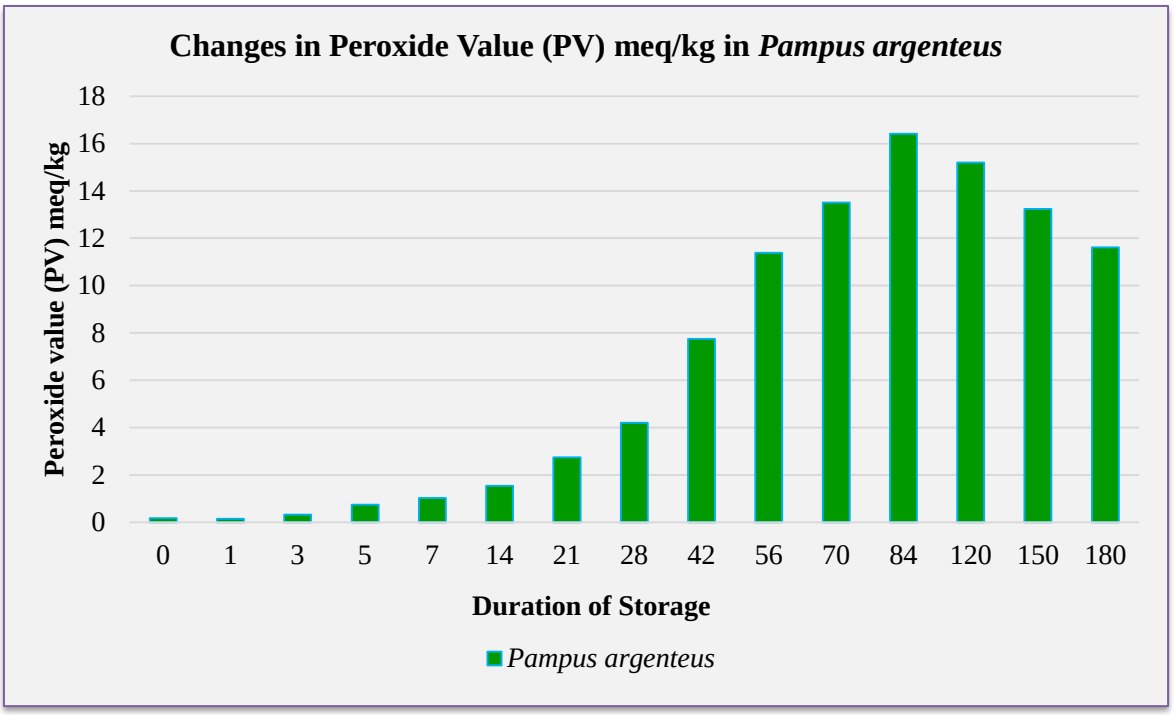

Figure 1. Changes in Peroxide Value meq/kg in *Pampusargenteus*

TBA content was not detected initially in fresh as well as after one day of frozen storage. After three days of frozen storage TBA value reached 0.10 mg malonaldehyde/kg. TBA content gradually increased and remained below the limit of acceptability (7–8 mg malonaldehyde/kg as proposed by Huss, 1988 until the completion of 120 day. Thereafter,

TBA values increased and have exceeded the limits of acceptability by the end of 150 days with a value of 9.45 mg malonaldehyde/kg.

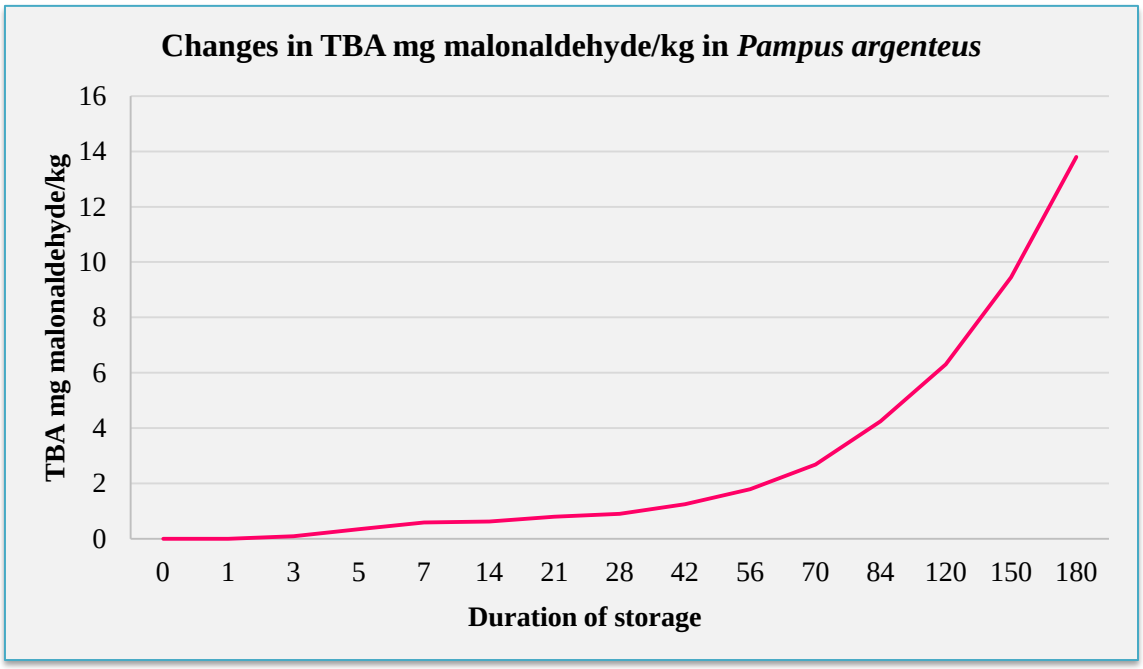

Figure 2. Changes in TBA value mg malonaldehyde/kg in *Pampusargenteus*

Discussion

Aquatic foods can retain their quality and nutrients over time by being frozen, which is a widely used technique. Low temperatures effectively stop microbial growth and enzyme activity, but they also cause protein and lipid oxidation, which can worsen physicochemical properties (Santos *et al.*, 2019), especially when there is temperature fluctuation and frequent freeze-thaw cycles (Nikoo, *et al.*, 2016). According to Huss (1995), fish lipid is rich in polyunsaturated fatty acids and therefore susceptible to oxidation. The development of rancidity during frozen storage was indicated by an increase in PV in frozen fish as compared to fresh fish (Ben-Gigirey *et al.*, 1999). One of the main impacts of lipid oxidation is the development of off-flavor; at a later stage of lipid peroxidation, changes in colour and nutritional value are seen as well (Dragoev *et al.*, 1998).

The impact of storage on the lipids and proteins in Atlantic mackerel that was kept at -20 and -30°C for up to 24 months was investigated. The results of the present study are well in agreement with the reports of Saeed and Howell, (2002) which stated that the peroxide value and thiobarbituric acid-reactive substances (TBARS) tests, among other conventional techniques, revealed an increase in lipid oxidation products with storage duration and at a higher storage temperature of -20 °C. The oxidative changes in frozen fish lipids may be caused by the occurrence of radicals, which are indicators of this process. According to Ben-Gigirey *et al.*,(1999), an increase in PV in frozen fish, as opposed to fresh fish, indicated the development of rancidity during frozen storage. Previous research (Liu *et al.*, 2010; Tokur *et al.*, 2004) have shown a similar pattern of increase in the TBA value. A similar observation of results is made in the present study also.

According to Lakshmisha *et al.*,(2008), fish may be consumed up to a level of 8 mg malonaldehyde/kg, with a maximum level of 5 mg malonaldehyde/kg indicating good quality

for fish that has been frozen, chilled, or stored with ice. Due to increasing oxidation and enzymatic hydrolysis of unsaturated fatty acids, lipid degradation is the primary factor in decreasing fatty acid shelf life (Sarma *et al.,* 2000).All samples of blue fin tuna had higher peroxide values after a year of storage, even if they were lower at lower temperatures (-45 and -60°C). All samples held at -20°C had higher peroxide values after three months (Tanaka *et al.,* 2016). TBA records revealed an increased rate of lipid oxidation during frozen storage of the Red Tilapia fillet samples. A significant increase in TBA (from 0.03 in fresh samples to 1.26) was observed at the end of the storage at -18^{o}C for a duration of 150 days. The highest value of PV was observed in 150th day (0.93 meq/kg) (Karami, *et al.,* 2013). During frozen storage, the shark and mackerel both showed a considerable increase in TBA, going from an initial value of 0.038 to 0.068 mg of malonaldehyde/kg of fish flesh and from 0.038 to 0.104, respectively. This showed oxidative degradation (Sahari *et al.,* 2009). According to Wu *et al.,*(2022), investigations were made into lipid oxidation in ice-stored sorted herring fractions from spring and autumn (head, backbone, viscera + belly flap, tail, fillet), as well as its relationships with endogenous prooxidants, antioxidants, and lipid substrates. They reported that after one day, the peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) had dramatically increased in all fractions.

The loss in fish freshness starts as soon as the postmortem and harvesting changes, highlighting the importance of good management from harvest through processing/commercialization to maintain the high quality and extended shelf life. According to the processing and preservation techniques used, this care would determine the oxidative, enzymatic, and microbiological activity in fish (Tian *et al.,* 2022). Several aspects connected to the organism (species, habitat, food, etc.) as well as the way of transportation to the preparation/processing business, which must also be taken into account while optimising freezing and refrigeration settings, have an impact on the shelf-life of fish (Walayat *et al.,* 2023).

Karacam and Boran, (1996) reported that the TBA value in anchovies stored at -18^{o}C increased significantly by the end of the storage period, indicating hydrolytic and oxidative changes. Ludoff and Meyer (1973) pointed out that in very fresh fish the peroxide value should be below 2 meq/kg fat, while in fresh fish the peroxide value should not exceed 5 meq/kg fat. The increase in TBA observed in the present study was probably due to destruction of hydroperoxides into secondary lipid oxidation products. Similar observations were previously made by (Aubourg *et al.,* 2002; Kolakowska, 2002)

Conclusion

Fish spoilage is caused by three main processes namely microbial growth, oxidation, and enzymatic autolysis. The most widely used methods in the market today for controlling water activity, enzymatic, oxidative, and microbiological spoilage are chemical methods and low temperature storage. Although energy-intensive freezing processes can reduce microbiological and enzymatic decomposition but unfortunately these processes cannot stop oxidative spoilage, they are only a temporary solution for fish preservation. To maximize the shelf-life of fish, further research is needed to understand the function of the fish's proximate composition, post-harvest history, ambient factors, initial microbial load, type, and nature of bacteria, and their interactions resulting in lipid oxidation.

Conflict of Interest

The author declares no conflict of Interest.

References

1. Alexandratos, N. and Bruinsma, J. 2012. World agriculture towards 2030/2050: the 2012 revision. ESA Working paper No. 12-03. Rome, FAO.
2. Aubourg, S.P., Lehmann, I. and Gallardo, J.M. 2002. Effect of previous chilled storage on rancidity development in frozen horse mackerel (*Trachurus trachurus*). Journal of the Science of Food and Agriculture, 82(15): 1764-1771.
3. Badii, F. and Howell, N.K. 2002. Changes in the texture and structure of cod and haddock fillets during frozen storage. Food Hydrocolloids, 16: 313–319.
4. Bayir, A., Haliloglu, H.Y., Sirkecioglu, A.N. and Aras, N.M. 2006. Fatty acid composition in some selected marine fish species living in Turkish waters. Journal of the Science of Food and Agriculture, 86: 163-168.
5. Ben-Gigirey, B., Desousa, J.M., Villa, T.G. and Barros-Velazqez, J. 1999. Chemical changes and visual appearance of albacore tuna as related to frozen storage. Journal of Food Science, 64: 20-24.
6. Cai, J. and Leung, P. 2017. Short-term projection of global fish demand and supply gaps. FAO Fisheries and Aquaculture Technical Paper (601), 607.
7. Dragoev, S.G., Kiosev, D.D., Danchev, S.A., Ioncheva, N.I., Genov, N.S. 1998. Study on the oxidative processes in frozen fish. Bulgarian Journal of Agricultural Science, 4: 55–65.
8. Egan, H., Kirk, R.S., and Sawyer, R. 1981. Pearson's chemical analysis of Food. 8th edition. Churchill Livingston. Longman group Limited.
9. Hocaoğlu, A., Demirci, A.S., Gümüs, T. and Demirci, M. 2012. Effects of gamma irradiation on chemical, microbial quality and shelf life of shrimp. Radiation Physics and Chemistry, 81(12): 1923-1929.
10. Huss, H.H. 1995. Quality and quality changes in fresh fish. FAO Fisheries Technical Paper 348, FAO: Rome, Italy.
11. Karaçam, H. and Boran, M. 1996. Quality changes in frozen whole and gutted anchovies during storage at −18 C. International Journal of Food Science and Technology, 31(6): 527-531.
12. Karami, B., Moradi, Y., Motallebi, A.A., Hosseini, E. and Soltani, M. 2013. Effects of frozen storage on fatty acids profile, chemical quality indices and sensory properties of red tilapia (*Oreochromis niloticus* × *Tilapia mosambicus*) fillets. Iranian Journal of Fisheries Sciences, 12(2): 378-388.
13. Kolakowska, A. 2002. Lipid oxidation in food systems. In: Sikorski, Z. and Kolakowska, A. Chemical and functional properties of food lipids, 2002. pp. 133-165. London (UK): CRC Press.
14. Kris-Etherton, P.M., Harris, W.S. and Appel, L.J. 2003. Omega-3 fatty acids and cardiovascular disease: new recommendations from the American Heart Association. Arteriosclerosis, Thrombosis, and Vascular Biology, 23(2): 151-152.
15. Lakshmisha, I.P., Ravishankar, C.N., Ninan, G., Mohan, C.O. and Gopal, T.K.S. 2008. Effect of freezing time on the quality of Indian Mackerel (*Rastrelliger kanagurta*) during frozen storage. Sensory and Food Quality, 73(7): 345-353.

16. Liu, S., Fan, W., Zhong, S., Ma, C.H., Li, P., Zhou, K., Peng, Z. and Zhu, M. 2010. Quality evaluation of tray-packed tilapia fillets stored at 0ºC based on sensory, microbiological, biochemical and physical attributes. African Journal of Biotechnology, 9(5): 692-701.

17. Ludoff, W. and Meyer, V. 1973. Fische und Fischerzeugnisse. Paul Parey. Hamburg, 95-111, 176-269.

18. Nikoo, M., Benjakul, S. and Rahmanifarah, K. 2016. Hydrolysates from marine sources as cryoprotective substances in seafoods and seafood products. Trends in Food Science and Technology, 57: 40-51.

19. Pazos, M., Gallardo, J.M., Torres, J.L. and Medina, I. 2005. Activity of grape polyphenols as inhibitors of the oxidation of fish lipids and frozen fish muscle. Food Chemistry, 92(3): 547-557.

20. Rodríguez Herrera, J.J., Pastoriza, L. and Sampedro, G. 2000. Inhibition of formaldehyde production in frozen-stored minced blue whiting (*Micromesistius poutassou*) muscle by cryostabilizers: An approach from the glassy state theory. Journal of Agricultural and Food Chemistry, 48(11): 5256-5262.

21. Saeed, S. and Howell, N.K. 2002. Effect of lipid oxidation and frozen storage on muscle proteins of Atlantic mackerel (*Scomber scombrus*). Journal of the Science of Food and Agriculture, 82(5): 579-586.

22. Sahari, M.A., Nazemroaya, S. and Rezaei, M. 2009. Fatty Acid and Biochemical Changes in Mackerel (*Scomberomorus commerson*) and Shark (*Carcharhinus dussumieri*) Fillets during Frozen Storage. American-Eurasian Journal of Sustainable Agriculture, 3(3): 519-527.

23. Santos, H.M.C., Méndez, L., Secci, G., Parisi, G., Martelli, R. and Medina, I. 2019. Pathway-oriented action of dietary essential oils to prevent muscle protein oxidation and texture deterioration of farmed rainbow trout. Animal, 13(9): 2080-2091.

24. Sarma, J., Reddy, G.V.S. and Srikar, L.N. 2000. Effect of frozen storage on lipids and functional properties of proteins of dressed Indian oil sardine (*Sardinella longiceps*). Food Research International, 33(10): 815-820.

25. Secci, G. and Parisi, G. 2016. From farm to fork: Lipid oxidation in fish products. A review. Italian Journal of Animal Science, 15(1): 124-136.

26. Sidhu, K.S. 2003. Health benefits and potential risks related to consumption of fish or fish oil. Regulatory Toxicology and Pharmacology, 38(3): 336–344.

27. Tanaka, R., Nakazawa, N., Maeda, T., Fukushima, H., Wada, R., Sugiura, Y. and Fukuda, Y. 2016. Effects of chilled storage, freezing rates, and frozen storage temperature on lipid oxidation in meat blocks from cultured bluefin tuna *Thunnus thynnus*. Journal of Aquatic Food Product Technology, 25(7): 1073-1085.

28. Tian, J., Walayat, N., Ding, Y. and Liu, J. 2022. The role of trifunctional cryoprotectants in the frozen storage of aquatic foods: Recent developments and future recommendations. Comprehensive Reviews in Food Science and Food Safety, 21(1): 321-339.

29. Tokur, B., Polat, A., Beklevik, G. and Özkütük, S. 2004. Changes in the quality of fishburger produced from Tilapia (*Oreochromis niloticus*) during frozen storage (−18^{0}C). European Food Research and Technology, 218: 420-423.

30. Von Schacky, C. 2000. n−3 fatty acids and the prevention of coronary atherosclerosis. The American Journal of Clinical Nutrition, 71(1): 224s-227s.

31. Vyncke, W. 1970. Direct determination of Thiobarbituric acid value in trichloroacetic acid extracts of fish as a measure of oxidative rancidity. Fette, Seifen, Anstrichmittel, 72: 1084-1087.

32. Walayat, N., Tang, W., Wang, X., Yi, M., Guo, L., Ding, Y. and Ranjha, M.M. 2023. Quality evaluation of frozen and chilled fish: A review. eFood, 4(1): e67.

33. Walayat, N., Xiong, H., Xiong, Z., Moreno, H.M., Nawaz, A., Niaz, N. and Randhawa, M.A. 2022. Role of cryoprotectants in surimi and factors affecting surimi gel properties: A review. Food Reviews International, 38(6): 1103-1122.

34. Wu, H., Forghani, B., Abdollahi, M. and Undeland, I. 2022. Lipid oxidation in sorted herring (*Clupea harengus*) filleting co-products from two seasons and its relationship to composition. Food Chemistry, 373: 131523.