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Lipid Oxidation Changes in Whole *Sphyraena barracuda* from Visakhapatnam Stored at -20^{o}C

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ABSTRACT
Lipid oxidation is one of the major causes of quality deterioration in fish and other seafood products, particularly in species that contain high levels of lipids. Marine fish are especially vulnerable to this process because their tissues are rich in highly polyunsaturated fatty acids (PUFAs), which are chemically unstable and easily react with oxygen. Although lipid oxidation can occur in many animal-derived foods, the high PUFA content in fish makes them far more prone to oxidative spoilage. This reaction leads to the formation of undesirable compounds that cause rancid flavors, off-odors, discoloration, and overall loss of nutritional and sensory quality.In this context, the present study was conducted to evaluate the extent of lipid oxidation in whole Sphyraena barracuda collected from the Visakhapatnam harbor during frozen storage at –20 °C. The progression of oxidation was monitored using two widely accepted chemical indicators of lipid deterioration: Peroxide Value (PV), which measures the formation of primary oxidation products, and Thiobarbituric Acid (TBA) value, which reflects the accumulation of secondary oxidation products such as malondialdehyde. The results of the study indicated progressive lipid oxidation over time. Based on these findings, it can be concluded that storing whole fish at temperatures lower than –20 °C would be more effective in slowing oxidative changes and maintaining acceptable quality for a longer storage period.
Keywords: Sphyraena, PV, TBA

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INTRODUCTION

Fish and shellfish have long held an important place in human diets, providing a significant share of dietary protein for many coastal communities around the world. In recent years, fish consumption has increased due to growing awareness of its health benefits, such as low cholesterol and fat levels and its high-quality animal protein. Improved distribution systems, greater advertising, and the recognition of fish as a nutritious food rich in essential minerals have also helped make fish a regular part of many people's diets. Fish and shellfish are considered valuable components of a healthy diet because they are excellent sources of Omega-3 polyunsaturated fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Studies suggest that consuming EPA and DHA can help prevent or reduce the risk of death from cardiovascular diseases, atherosclerosis, aging, and certain types of cancer, especially in individuals who have already experienced cardiac problems [18, 2, 6, 25, 22, 5]. Furthermore, long-chain polyunsaturated fatty acids (PUFAs) are now regarded as "conditionally essential" nutrients for proper growth and development in infants [33, 10].

Fish and other aquatic products constitute an essential component of the human diet due to their high nutritional value and significant health benefits. Fish typically contains approximately 15–20% high-quality protein and provides essential amino acids required for human growth and maintenance. In addition to protein, fish supplies important micronutrients such as vitamins A, D, E, K, B_{12}, and minerals including iodine, fluorine, and chlorine, which contribute to the prevention of nutritional deficiencies and support overall health. Marine fish are also rich in omega-3 polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have been associated with cardiovascular protection, improved neurological development, and reduced risk of chronic diseases [27, 28, 16].

Beyond their nutritional importance, fish and fishery products play a crucial role in global food security and economic development. The fisheries sector supports livelihoods across a wide range of activities, including capture fisheries, aquaculture production, processing, distribution, and marketing. Every year, the amount of land available for cultivation keeps shrinking. This happens for many reasons, including climate change, natural disasters, expanding industries, the need to use land for other purposes, and sometimes poor farming or grazing practices. As a result, it is becoming increasingly important to protect the food resources we already have and find ways to reduce food waste.

Advances in processing technologies and preservation techniques have facilitated the international trade of fish products, allowing them to be distributed globally in fresh, chilled, frozen, and processed forms [23]. Additionally, fish and fish by-products are widely utilized in the production of animal feed, oils, fertilizers, and pharmaceutical compounds, further highlighting their economic significance [7].

Despite these advantages, fish is highly perishable due to its biochemical composition. Fish muscle typically contains high moisture content, abundant unsaturated lipids, and active endogenous enzymes that accelerate spoilage after harvest. These characteristics make fish particularly susceptible to biochemical and microbial deterioration during storage and distribution [1, 8]. Without appropriate preservation methods, rapid degradation may lead to significant losses in quality, nutritional value, and consumer acceptability.

Among the available preservation techniques, freezing is widely regarded as one of the most effective methods for extending the shelf life of fish and other aquatic products. Freezing lowers the temperature of fish tissues and converts most of the water into ice, thereby significantly reducing microbial activity and slowing enzymatic and biochemical reactions responsible for spoilage. As a result, frozen storage enables long-term preservation and facilitates the global distribution of fish products [16, 27]. The quality of fish often declines during frozen storage because of undesirable changes that occur in its lipids and proteins. These changes are commercially significant because they directly affect how long frozen fish and shellfish can be stored while maintaining acceptable quality [10]. Both temperate and tropical fish tend to deteriorate during storage due to several factors, including microbial spoilage, the activity of natural enzymes within the fish, non-enzymatic lipid oxidation, and browning reactions [20, 31, 13-15, 9, 17, 11, 3, 9]. As these processes occur, the fish gradually loses its desirable color, smell, and flavor, which ultimately reduces its overall quality and shelf life [4, 29, 30].

Thus, freezing does not completely stop biochemical reactions in fish muscle. During frozen storage, several deteriorative processes may continue to occur, including lipid oxidation, protein denaturation, moisture loss, and structural damage caused by ice crystal formation. These changes can negatively affect important quality attributes such as color, flavor, texture, and water-holding capacity, ultimately reducing the sensory and nutritional quality of fish products [23, 26].

Lipid oxidation is considered one of the primary mechanisms responsible for quality deterioration in frozen fish. The oxidation process begins with the formation of lipid hydroperoxides as primary oxidation products. These unstable compounds subsequently decompose into secondary products such as aldehydes, ketones, and short-chain fatty acids, which contribute to rancid odors, off-flavors, and reduced consumer acceptability [12]. In addition to lipid oxidation, protein oxidation also plays a significant role in frozen fish deterioration. Oxidative reactions can lead to the formation of carbonyl compounds, disulfide bonds, and protein aggregates, resulting in decreased protein solubility, reduced water-holding capacity, and impaired functional properties of fish muscle proteins [26].

Due to these complex biochemical processes, numerous studies have investigated the effects of frozen storage on fish quality using various physicochemical and biochemical indicators. Parameters such as peroxide value (PV), thiobarbituric acid (TBA), free fatty acids (FFA), total volatile nitrogen (TVN), and trimethylamine (TMA) are commonly used to evaluate lipid oxidation, lipid hydrolysis, and protein degradation in fish during storage [28, 1]. Maintaining the quality of fish during storage is essential to ensure that it remains safe, nutritious, and appealing to consumers. Therefore, proper preservation and handling methods are necessary to slow down deterioration and extend the shelf life of fish and shellfish products. Understanding these changes is essential for improving preservation strategies and maintaining the quality and safety of frozen fish products during storage and distribution.

MATERIAL AND METHODS

The present study includes studies on Peroxide value and TBA measurements of one of the marine species namely *Sphyraena barracuda* landed at Visakhapatnam fishing harbour. 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. 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 water bath for not more than 30 seconds. 30 ml of water was added slowly 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

Many studies have reported progressive lipid oxidation in fish during frozen storage. Lipid oxidation is commonly evaluated using peroxide value (PV) and thiobarbituric acid (TBA) value, which represent primary and secondary oxidation products, respectively. In the present study, initial PV in fresh *Sphyraena barracuda* was 1.01 meq/kg. After one day of frozen storage PV increased and reached 1.09meq/kg. With a gradual increase PV reached 12.50meq/kg after 70 days. On further storage PV decreased and reached 8.90meq/kg after 180 days of frozen storage. Results showed that fresh *S. barracuda* had no TBA content. After one day of frozen storage TBA value 0.08 mg malonaldehyde/kg was detected. Gradual increase was observed for the entire storage period. After 70 days TBA value recorded was 5.75 mg malonaldehyde/kg. Further storage led to an increase in TBA value that exceeded the limit of acceptability (7-8 mg malonaldehyde/kg) proposed by Huss [9]. Thus, after 180 days of storage TBA value reached 11.75 mg malonaldehyde/kg.

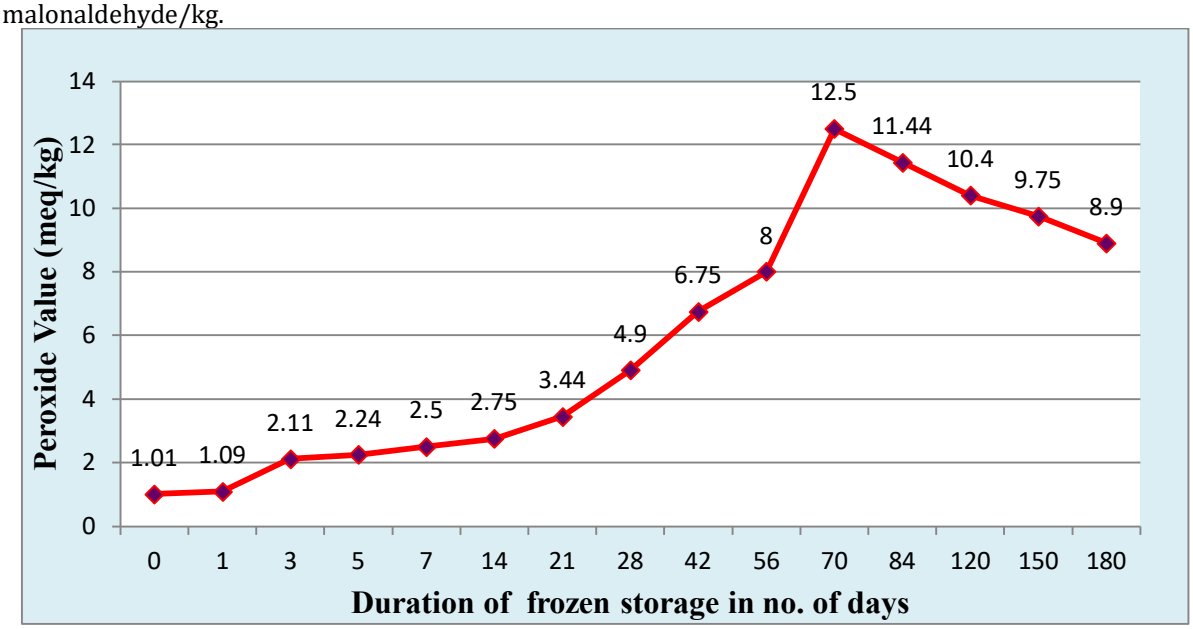

Fig 1: Peroxide Value (meq/kg) in *S. barracuda*

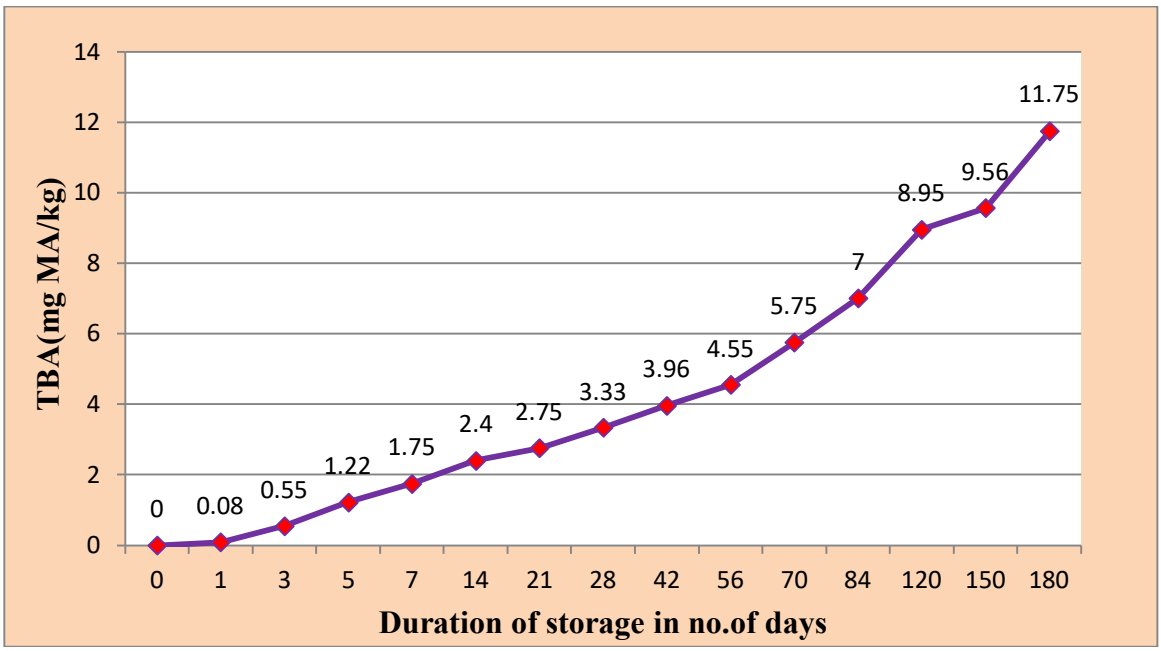

Fig 2: TBA (mg MA/kg) in *S. barracuda*

In frozen fillets of *Psenopsis cyanea*, PV increased from 3.32 to 28.82 meq/kg over 36 weeks of storage, indicating a gradual accumulation of primary oxidation products. Similarly, TBA values increased from 0.48 to 2.9 mg malonaldehyde/kg, reflecting the formation of secondary oxidation products associated with rancidity development [24].

Similar trends were observed in several fish species from the South Caspian Sea. During six months of frozen storage at −24 °C, PV increased significantly in all species studied. PV in pike perch increased from 4.29 to 33.13 meq O_2/kg lipid, demonstrating progressive oxidative deterioration during storage. TBA values showed species-specific peaks during storage before declining slightly, likely due to interactions between malonaldehyde and muscle proteins [27].

In shortfin scad (*Decapterus macrosoma*), lipid oxidation progressed more rapidly during chilled storage than frozen storage. PV increased from 0.38 to 1.54 mEq/kg in chilled fish compared with 1.15 mEq/kg in frozen samples, while TBA values reached 5.46 mg/kg in chilled samples but only 1.06 mg/kg in frozen samples [21]. These results demonstrate that freezing significantly slows lipid oxidation compared with chilled storage.

Studies evaluating imported frozen fish species also reported measurable but acceptable levels of lipid oxidation. For example, TBA values ranged between 0.74 and 1.82 mg malondialdehyde/kg in several species marketed in Basrah markets, indicating moderate lipid oxidation during storage [23].

DISCUSSION

The studies reviewed collectively demonstrate that frozen storage significantly slows but does not completely prevent biochemical and structural deterioration in fish muscle. Quality changes during frozen storage result from the combined effects of lipid oxidation, protein denaturation, enzymatic reactions, and physical damage caused by ice crystal formation. Lipid oxidation appears to be one of the most important factors influencing the deterioration of frozen fish quality. The increase in peroxide value observed in many studies indicates the formation of lipid hydroperoxides during storage. These unstable compounds eventually decompose into secondary oxidation products such as aldehydes and ketones, which contribute to rancid odors, off-flavors, and reduced sensory quality of fish products [12, 27].

Lipid hydrolysis also plays an important role in frozen fish deterioration. The accumulation of free fatty acids reflects the enzymatic breakdown of lipids during storage. Although free fatty acids themselves may not directly cause quality loss, they can accelerate oxidative reactions and contribute to undesirable changes in flavor and texture [28].

Physical damage caused by ice crystal formation also contributes to quality deterioration in frozen fish. Ice crystals disrupt cellular structures and muscle fibers, leading to protein denaturation and reduced water-holding capacity. Ice crystal formation during frozen storage of fish can indirectly influence lipid oxidation. When fish is frozen, particularly under slow freezing conditions, large ice crystals may develop within the muscle tissues. These crystals can disrupt cellular structures, rupture cell membranes, and damage muscle fibers, which exposes lipids that were previously protected within cellular compartments to oxygen and

other pro-oxidative agents. This increased exposure makes lipids more susceptible to oxidative reactions. Moreover, the structural damage caused by ice crystals can lead to the release of pro-oxidative components such as heme proteins and metal ions from intracellular sites, which can catalyze lipid oxidation processes. In addition, during freezing, the formation of ice concentrates solutes in the remaining unfrozen phase, creating conditions that may further promote oxidative reactions. Therefore, although ice crystal formation primarily causes physical deterioration of fish muscle, it also indirectly accelerates lipid oxidation by enhancing lipid exposure and promoting pro-oxidative conditions in the tissue [32, 34, 21, 33]. As a result, thawing loss increases and fish muscle becomes softer and less elastic during storage [21].

The findings indicate that frozen storage remains an effective method for preserving fish quality, but its effectiveness depends on several factors, including species composition, storage temperature, storage duration, and handling conditions. Maintaining stable freezing temperatures and minimizing oxidative reactions are essential for preserving the nutritional value, sensory quality, and shelf life of frozen fish products.

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