STUDIES ON
FREEZING AND STORAGE PERFORMANCE OF TILAPIA FILLETS

THESIS

Submitted in partial fulfillment of the requirements

for the Degree of

MASTER OF SCIENCE
IN
POST HARVEST MANAGEMENT OF MEAT, POULTRY AND FISH

By
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NOVEMBER, 2022

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DECLARATION OF STUDENT

I hereby declare that the experimental work and its interpretation of the Thesis entitled **"STUDIES ON FREEZING AND STORAGE PERFORMANCE OF TILAPIA FILLETS"** or part thereof has neither been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis/publication of any University or scientific organisation. The source of materials used and all assistance received during the course of investigation have been duly acknowledged and that no part of the thesis has been submitted for any other degree or diploma

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CERTIFICATE

This is to certify that the thesis entitled, **"STUDIES ON FREEZING AND STORAGE PERFORMANCE OF TILAPIA FILLETS"** submitted for the degree of M. Sc. (Agri.) in Meat,Poultry,Fish of the College of Post Graduate Institute of Post Harvest Technology and Management, Killa-Roha, is a bonafide research work carried out by **Mr. Pratik Kiran Bhujbal** under my supervision and that no part of this thesis has been submitted for any other degree. The student had completed all the Course and Research requirements as per the norms in regular mode.

The assistance and help received during the course of investigation have been fully acknowledged.

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This is to certify that the thesis entitled, **"STUDIES ON FREEZING AND STORAGE PERFORMANCE OF TILAPIA FILLETS"** submitted by **Mr. Pratik Kiran Bhujbal (PHMRM0220175)** to the College of **Post Graduate Institute of Post Harvest Technology and Management, Killa-Roha**, in partial fulfillment of the requirements for the degree of M.Sc. (Pratik Kiran Bhujbal)in the subject having **Food Hygiene and Quality Control** as Minor subject of **Department of PHM of Meat,Poultry,Fish** has been approved by Student's Advisory Committee, Board of Studies of the Department and Evaluated by One External Examiner after an open Viva Voice examination in the presence of External Examiner on the same held on dated / / 2022.

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Place: - Killa-Roha
Date: / /2022

*(***Mr.Pratik Kiran Bhujbal***)***

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ABBREVIATIONS

%	Percent
a*	Redness
AOAC	Association of Official Analytical Chemists
Avg	Average
b*	Yellowness
C.D.	Critical difference
C.V.	Coefficient of variation
CBC	Complete blood cell count
CFCD	China Food Composition Database
CFU	Colony Forming Unit
CuSO4	Copper sulphate
˚C	Degree celsius
E-coli	Escherichia coli
F.C.R.D	Factorial completely randomised design
FAO	Food & Agriculture Organisation
FFA	Free fatty acids
GIFT	Genetically improved farmed tilapia
gm	Grams
hrs	Hours
IQF	Individual Quick Freezing
L*	Lightness
mg	Milligram
N.D.	Not detected
N.S	Non significant
NaCl	Sodium Chloride
NaOH	Sodium hydroxide pellets
NPN	Non protein Nitrogen
P.V	Peroxide value
pH	Potential of Hydrogen
PUFA	Polyunsaturated fatty acids
S.Em	Standard error of mean
SDS-PAGE	Sodium dodecyl-sulfate polyacrylamide gel electrophoresis
sp	Species
STPP	Sodium tripolyphosphate
T	Treatment
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid-reactive substance
Temp	Temperature
TMA	Trimethylamine oxide
TMAO	Trimethylamine oxide
TPC	Total Plate Count
TVB-N	Total Volatile Base Nitrogen
TVN	Total volatile nitrogen
WHC	Water holding capacity

GLOSSARY

Aquaculture- The farming of freshwater and saltwater organisms including molluscs, crustaceans and aquatic plants.

Freezing - Extracting heat and reducing the temperature of fish in a freezer to a point at which almost all of the water in it has solidified

Glazing -Applying a protective coating of ice, an ice glaze, to unwrapped frozen fish, fillets, t crustaceans, adductor muscle of scallops or other suitable frozen fish product, to reduce undesirable drying or dehydration of the fish during frozen or cold storage

Grading - The allocation of a defined category, or grade, of quality to a sample, or collection of similar samples, of a product.

Individual quick freezing (IQF) - It is the quick freezing of fish products, e.g. fillets, in such a manner that each unit of product remains separate when frozen, in contrast to a frozen block of product

Moisture- Synonymous with water content in the context of proximate composition.

Proximate composition - The main constituents of a product, and their amounts in unit mass or volume. In the case of products without added components, proximate composition typically comprises water, protein, fat, and ash, expressed as percentage of the weight.

Total Volatile Basic Nitrogen (TVBN) - The amount of basic, nitrogen-containing chemicals distilled from an alkalised extract or suspension of a fishery product.

Ash - The residue remaining after all the organic matter in a sample has been burned off. Often included in the proximate composition of a product.

Frozen storage - A refrigerated and insulated room, suite of rooms, or a large refrigerated and insulated enclosed space, on land or in a ship, for preservation of fish and fish products in the frozen state

Drip loss - It is the amount of liquid that is lost and is usually expressed as a percentage of the original

CHAPTER I: INTRODUCTION

1.1 Background information

Seafood has a great nutritional value due to its abundance in necessary amino acids, vital fatty acids, minerals, and vitamins, as well as its low content in saturated fats and cholesterol. One of the most significant sources of animal protein in the tropics is fish, which is also widely acknowledged as an excellent source of other nutrients for the maintenance of a healthy body (Andrew, 2001).50% of the global catch is caught by less developed nations, and most of the catch is consumed domestically. Fish makes up more than 50% of the animal protein consumed in many Asian countries, but only 17.50% in Africa (Williams et al., 1988). Fish makes over 40% of the animal protein consumed in Nigeria Fish is a significant source of high-quality proteins, vitamins—particularly vitamins A, E, and D—as well as minerals including potassium, salt, calcium, magnesium, iron, copper, zinc, and manganese. (Olatunde, 1998)

Fluorine and iodine, which are necessary for the growth of healthy teeth and the prevention of goitre in men (an enlargement of the thyroid gland located in the neck), are found in fish, which is a rich source of both (Andrew, 2001). However, the techniques of storage, such as salting, roasting, drying, and freezing, have a significant impact on the availability of these essential nutrients (Hardy and Smith, 1976; Botta et al., 1978; Ryder et al., 1993). Fish quality loss and shelf life are mostly influenced by storage time and temperature (Whittle, 1997).Fish is a significant dietary source in many nations and is highly recognised for its nutritional value. Polyunsaturated fatty acids (PUFAs) are abundant in fish lipids, especially eicosapentaenoic and docosahexaenoic acids (EPA; 20:5n-3 and DHA; 22:6n-3, respectively) (Pazos et al., 2005; Bayir et al., 2006).Due to the superior product quality, fish has been preserved by freezing for thousands of years (Persson and Londahl,1993).

Fillet comes from the french word filet pronounced(filɛ) meaning a thread or strip, is the meat of a fish which has been cut or sliced down from the bone by cutting lengthwise along one side of the fish parallel to the backbone. In medication for filleting, any scales on the fish should be removed. The contents of the stomach also need careful detaching from the fillet. Because fish fillets don't contain the larger bones running along the spine, they're frequently said to be" boneless". Still, some species, similar to the common carp, have lower intramuscular bones called legs within the fillet. The skin present on one side may or may not be stripped from the fillet. Butterfly fillets can be produced by cutting the fillets on each side in such a way that they're held together by the meat and skin of the belly.Fish fillets can be varied with fish steaks (also known as fish croquettes), which are cut vertical to the spine and include the larger bones.(Murray and Burt,1983).

Fish fillets comprise the meat of the fish, which is the cadaverous muscles and fat as opposed to the bones and organs. Fillets are generally attained by slicing the fish parallel to the spine, rather than vertical to the spine as is the case with steaks. The remaining bones with the attached meat is called the" frame", and is frequently used to make fish stock. As opposed to whole fish or fish steaks, fillets don't contain the fish's backbone; they yield lower meat, but are easier to eat. Special cut fillets are taken from solid large blocks; these include a" natural" cut fillet, wedge, rhombus or tail shape. Fillets may be skinless or have skin on; leg bones may or may not be removed.

There are several ways to cut a fish fillet :-

- Cutlet- Attained by slicing from behind the head of the fish, round the belly and tapering towards the tail. The fish is also turned and the process repeated on the other side to produce a double fillet
- Single- More complex than the croquette, produces two separate fillets, one from each side of the fish.
- " J" Cut- Produced in the same way as a single fillet but the leg bones are removed by cutting a" J" shape from the fillet. (Green & Aliza, 2010).

When a fish dies, the immune system disintegrates, allowing bacteria to flourish unchecked (Huss, 1988). During handling and processing, the flesh becomes quickly infected by intestinal and surface germs, as well as bacteria from tools and people. The majority of the microbial contamination in fish fillets is discovered to originate during filleting and subsequent handling before packaging (Adams and Moss, 2008). Because it raises the surface area to the volume of the product, opening the fish muscle through filleting generates circumstances for the invasion of germs and oxidation in fatty fish (Adams and Moss, 2008). Fish fillets are inevitably contaminated by microbes during processing, making it difficult for them to last for an extended period of time even when refrigerated (Whittle et al., 1990; Duun and Rustad, 2007). In order for the thawed fish to keep its freshness, frozen storage relies on reducing the product's temperature to prevent spoiling. The temperature of the product plays the biggest role in extending the shelf life because fish is more delicately perishable than meat. Fish from both marine and aquaculture origins can be refrigerated using freezing-point storage, which regulates the temperature between 0°C and the fish's freezing point. The freezing point of tilapia is about -7°C, according to (Chen and Pan,1995). The value of fish depends on how fresh it is, and as its exportability decreases, its look, flavour, and other quality of flavour, texture, and consumer acceptability declines (Kagawa et al. 2002).

Table 1.1:-Taxonomy of Tilapia (Linnaeus,1758) **:-**

Kingdom	Animalia
Phylum	Chordata
Class	Teleostei
Order	Perciformes
Family	Cichlidae
Tribe	Tilapiini
Genus	*Oreochromis*
Species	*Oreochromis niloticus*

Tilapia is the most popular and commonly grown species of farmed fish, coming in second only to carp (El-Sayed, 2006). It is grown in at least 85 nations, with China and Latin America (Ecuador, Honduras, and Costa Rica) producing the most of it. Due to its rapid growth rate, ease of cultivation, and high feed efficiency ratio, tilapia is one of the most popularly cultivated freshwater fish species worldwide (El-Sayed, 2006).Over 100 nations currently cultivate tilapia. Tilapia are often grown in ponds using comprehensive, semi-intensive, and intense production techniques. Rapid growth, high change tolerance, adaptation to a variety of environments with varying salinity and dissolved oxygen, resistance to stress and disease, captive reproduction, brief gestation periods, feeding from low nutrition levels, and accepting artificial food right away after absorbing the yolk sac are the main causes of the high level of tilapia production.Fishery production in 2008 was led by the provision of fresh goods (56 million ton), then by the supply of frozen fisheries was 29 million ton.Sugunan(1995) reported that the tropical reservoirs in India are an appropriate habitat for (*Oreochromis Mossambicus*), and there are self-sustaining populations of the species in most of the reservoirs in southern India.

Genetically improved farmed tilapia (GIFT) strain was developed back in 1988. At the time, tilapia husbandry had issues including poor productivity, deteriorating performance and shy force of seed.To address these issues, WorldFish, also known as the International Center for Living Submarine coffers Management or ICLARM, worked with mates from the Philippines and Norway to produce a briskly- growing strain of Nile tilapia that's suitable for monoculture, both small- scale and marketable in collaboration with mates, WorldFish innovated a methodical breeding system based on breeding programs for salmon and trout established in Norway in the 1970s.

Full sibling families – brother and sister seed from the same parents – are bred and also grown in separate cloth net coops until they're big enough to be collectively tagged with Passive Integrated Transponders(hole markers). They will also be transferred to a collaborative pond to be grown with seeds from other parents. The performance of all fish within the collaborative pond is covered collectively and fish from the best- performing families were also named as parents of the coming GIFT generation.WorldFish has demonstrated that this method is a doable, cost-effective and sustainable approach to the inheritable enhancement of tilapia. The GIFT strain is a result of 28 times of breeding across 23 generations. It's being used in 17 countries around the globe.The faster growth of the GIFT strain allows for a shorter product cycle and increased yield for the same plot of land, leading to reduced costs and increased profit. Also (GIFT), is one of the important species for aquaculture in India.(Trinh And Benzie,2022)

Individual Quick Freezing (IQF) - Common examples of IQF foods are fruits like blueberries, strawberries, and peaches; vegetables like sludge, peas, and green sap; seafood like shrimp and scallops; or flesh, like individual funk guts. Indeed whole flesh, similar to firmed clunkers, are reused using the IQF system. Also called flash- freezing, the IQF system involves transferring the individual food particulars on a kind of conveyor belt into a blast bite that freezes the item veritably snappily. Because the food particulars are separate when they go in, they stay separate after they have been firmed. The process was developed by a biologist Clarence birdseye while ice fishing in canada, noticed that the fish he pulled out of the water beneath the ice would incontinently indurate solid when he tossed them on the snow. Indeed more remarkable, still, was the fact that some of these fish would still be alive after they fused. This surprising result has to do with the fact that when a commodity freezes it forms lower ice crystals than when it freezes slowly. Specifically, this is because ice crystals only form between 31° F and 25 °F. The longer a food item spends in this temperature range, the further ice crystals will form.The key to IQF is speeding the food through this 31°F to 25°F temperature zone as snappily as possible.(Dailo,2016)

Quick freezing is at present the only process whereby virtually all the properties of most foodstuffs can be preserved. The important feature of this process is ultra-rapid freezing to very low temperatures (-30°C to - 40°C) designed to halt the activities of the microorganisms that cause decay and deteriorative activities in foodstuffs. I.Q.F is the latest technology available in freezing and with the advent of the same, it is now possible to preserve and store for more than a year, with the colour, flavour and texture of produce remaining as good as fresh. In IQF, each piece is frozen individually using technique of fluidization resulting in freezing only in 10 to 12 minutes which otherwise takes at least 3 to 4 hours or even more in the blast freezer. This results in a better texture and there is no lump/block formation and the product is free flowing. One does not have to thaw or defrost the whole packet to take out only a portion, and the rest will remain frozen till required again.

This system involves the use of a blast of cold air which, when directly on the food products, quickly freezes them. They are also frozen in an air blast tunnel (chamber freeze) in which cold air at –40°C at 2-5m/s is rapidly moved around the product giving it a cryogenic shock and freezing it instantly. This type of freezing results in the product free rolling and not clotting into lumps.(Pruthi 1995).

Air blast freezing - It is the process of taking a product at a temperature(generally chilled but occasionally at ambient temperature) and freezing it between 12 and 48 hrs, to its asked storehouse temperature which varies from product to product(e.g. fish = −20 °C, beef = −18 °C). Generally, the evaporator temperature in a blast freezer refrigeration system ranges between −35°C and −52°C. Slow freezing produces large ice chargers, which grow through cell walls, permitting an accelerated penetration of oxygen, causing rancidity and browning of meat and enhancing the peril of advanced drip on thawing. Thus, rapid-fire freezing is needed to maintain food quality as it produces small ice crystals due to an advanced number of nucleation points from which ice chargers form. Air Blast freezing is classified as a forced convection miracle where the use of suckers increases the products face heat transfer measure and produces a more invariant air temperature throughout the freezer. The air haste, and hence heat transfer measure, can be altered with the use of variable speed drives(VSD's). The main detriment of forced convection in blast freezers is the use of large suckers that add significantly to the total heat cargo on the refrigeration system and running costs. Also, unwrapped foods are prone to humidity loss during blast freezing as the absolute moisture of the bulk air is generally lower than that of the air at the face of the food.They have been used in industries since the 1950's.(Bansal et.al.,2012).

Blanching - It is a unit operation previous to freezing, canning,drying in which substances are hotted for the purpose of inactivating enzymes; modifying texture; conserving colour, flavour, and nutritive value; and removing trapped air.Water blanching is performed in hot water at temperatures ranging generally from 70 °C to 100 °C.Water blanching usually results in a more invariant treatment, allowing processing at lower temperatures. There are water blanchers that use a screw or a chain conveyor to transport the product inside the tank, where hot water is added.Others use a rotary barrel to immerse and convey the product. Water is generally hotted laterally with brume in a heat exchanger; thus brume quality doesn't need to be ‘‘ food-grade. ’’ Also it results in increased filtering of minerals and nutrients similar to vitamins, and produces effluents with large natural oxygen demand. (Dekker, 2014)3

1.2 Importance and need of study

Fish freezing is one of the optimal practices for preserving the natural nutritional and sensory qualities that the industry has employed extensively for a longer period of time.Fresh fisheries goods often have a short shelf life because to microbial activities, which are greatly impacted by storage temperature (Huss, 1995; Simpson et al., 2003).According to the species, harvest area, and season, the typical shelf-life of fish fillets in refrigerator storage settings ranges

from 2 to 14 days, which can cause significant financial loss (Wilhelm, 1982;Sivertsvik et al., 2002). Additionally, customers have higher expectations for continuously good food quality and expect that this quality will be upheld throughout the time between food production and consumption (Jones and Disney, 1996;Creed and Pierson, 1999).

In most of the western countries, frozen food products dominate the local market. There is increasing consciousness towards health and nutrition in India and hence the acceptance and consumption of IQF is likely to increase in India and abroad. Particularly in India,there is a great potential for utilising such techniques and technology to avoid deterioration of fresh commodities and convert them into value added products. The trade estimates for the industry in India project total production between 35,000 - 40,000 MT per annum valued at 250-300 crores annually. According to the US International Trade Administration, the frozen foods market size in India is US$ 9 million.Freezing fish and fisheries can help maintain the quality, extend shelf life, send seafood to markets with high consumption, and supply surplus fisheries throughout the year.However, significant disadvantages exist, including quality loss, weight loss, lipid oxidation, particularly in fatty fish, and high freezing costs.Despite a few drawbacks, freezing fish is recognised as an efficient method of fish preservation.For thousands of years, people have preserved food by freezing it because it keeps the products quality. Because freezing halts chemical and microbiological degradation, it is a superb method of preservation and is the best way to preserve commercial fish.(FAO,2008)

Tilapia is a crucial species for aquaculture and is expanding appeal as a quality alternative to animal sources of protein for use in human food due to its rapid growth and resistance the ability to breed in captivity, resistance to disease and handling ability to adapt to low-protein diets and endure a variety of spectrum of environmental variables (Shiau and Shue 1989).Tilapia is an affordable source of protein, vitamins, minerals and essential adipose acids that are vital for good health.Products made from tilapia come in a wide variety of international marketplaces.It has become a fish of choice because it's fast growing and an affordable source of protein. Tilapia is the third most important fish after beef and salmon in the world.This fish is most suitable for culture in tropical zones as the temperatures are largely suitable for fast growth. This fish can tolerate temperatures of 82-86°F.This fish is a fat breeder and sustained by several exploration institutions led to the product of mono sex male culture. This species takes only 6 months to reach 600- 900 gms from 50- 80 gm size.

Tilapia fillets are now offered in a variety of sizes and packaging options, including skin-on, skin-off, deep-skinned, individually quick frozen, smoked, and sashimi grade. They are also treated with carbon monoxide or ozone dipped. There have been some intriguing byproducts developed, including leather goods for apparel and accessories, time-released medication manufactured from skin gelatin, and floral decorations made from dried and coloured fish scales.(FAO,2008)

Retailers are looking for better freshness and a longer shelf life due to the rising demand for tilapia fillets. Consumers simultaneously anticipate high-quality food, with expectations of high-level quality maintenance between food production and consumption.The second most commercially farmed fish in the world is tilapia. They are raised on farms in at least 85 nations, with Asia and Latin America producing the majority (Eknath et al., 2007). China produced 1,210,000 tonnes of tilapia in 2007, accounting for almost 49% of the world yield (Li and Cai, 2008). About 66.7% of the tilapia produced in China is sold live, with the remainder being frozen for export or going through additional processing (Li and Cai, 2008). The low production of chilled tilapia is mostly caused by both its high perishability and inadequate quality assessment system. Numerous studies on tilapia have a focus on breeding performance and variety enhancements in particular (Yi and Lin,2001;Eknath et al,2007).Some studies on the storage quality of frozen or chilled tilapia (Eaves et al., 1995;Korel et al., 2001; Arannilewa et al., 2005; Yanar et al., 2006; Sil et al.,2008) were still insufficient in terms of the assessment of quality and the mechanisms of spoiling.Fishery goods are among the food categories that are traded worldwide on the global market in terms of how frequently they cross borders. Around 130 million tonnes of the total annual production are traded internationally. Furthermore, the consumer countries' increasingly rigid safety standards and laws provide difficulties for the nations that export fish. As a result, the fishing industry is logically becoming more concerned with food safety and quality.(Eknath et al.,2007)

1.3 Objectives :-

The present study was undertaken for following objectives as follows :-

1.Comparison of IQF and blast frozen storage performance of tilapia fillets

2.Evaluation of quality during frozen storage of tilapia fillets

1.4 Hypothesis or assumptions

The research was conducted on Tilapia (*Oreochromis niloticus)* to study freezing performance with the help of Individual quick freezing and blast freezing with different pretreatments like NaCl, STPP(Sodium tripolyphosphate),blanching with assumption to control microbial load and improving the physical, chemical and biochemical characteristics. With this assumption the research is conducted and results are presented in the thesis.

1.5 Scope and Limitations

1.5.1 Scope

The main factor affecting how long fresh fishery goods will stay fresh in the market is microbial activity, which is significantly influenced by the temperature of the storage facility. Thus, the food industry searches for strategies to prolong the freshness

of various fish species. Historically, fish sensitivity and nutritional values have been preserved through freezing and frozen storage.

Global shifts in consumer culture, marked by a growing desire for nutritious and healthy food products, is the cause of rise in demand for ready-to-cook fish and fisheries products. It is necessary to adopt preservation techniques that can raise value and reduce postharvest losses one of which is frozen storage.

1.5.2 Limitations

Unlike other foreign countries there is less consumption of frozen food in India. Most Indian people prefer fresh caught fish compared to frozen stored fish. Also in our country there are less facilities of freezing due to their high initial cost of freezing and maintenance of fish.

CHAPTER ll : REVIEW OF LITERATURE

2.1 INTRODUCTION

Fish is a highly perishable product with significant post-harvest losses.If it wasn't prepared and preserved, the fish decomposed very quickly.Fish bacteria, and enzymes are more responsible for fish deterioration. Preservation can simplify fish export to high-value markets and prevent wastage.When left untreated, fresh fish can contain up to 80% water by mass, which adds to microbial deterioration and a shorter shelf life (Bala and Mondol, 2001). Some techniques used to preserve food include freezing, canning, pickling, curing, smoking, and drying. Because fish is a marine product and is highly perishable, it is processed using freezing, canning, salting, and dehydration (Posomboon, 1998).

Measurement of fish spoilage was addressed by Beatty and Gibbons (1937). According to their research, the increase in volatile nitrogenous bases in codfish muscle between the pre-rigor stage and the odour's initial appearance is about 6 mg per 100 grams of tissue and is nearly completely the result of bacterial action. The outcome demonstrated that the increase as spoiling progresses is fifteen to twenty times the original value. Fish that have been preserved as well as untreated fish are both susceptible to spoilage.Due to its high water activity,high protein content, neutral pH, and presence of autolytic enzymes that cause fish to decay, fish is very perishable. Numerous factors, including species, fat content, fishing and slaughter techniques, hygienic manipulation, postmortem handling, and others, influence how quickly fish spoils Fish that have been postmortem goes through four stages: rigour mortis, rigour mortis resolving, autolysis, and bacterial deterioration.(Huss, 1995).

Endogenous autolytic enzymes in muscle are responsible for the initial loss of freshness, while microbial activities particularly the fast proliferation of specific spoilage organisms (SSO) are typically responsible for the subsequent deterioration (Huss,1995). Fish quality is accelerated by interactions between physicochemical processes and microbial metabolism. Deterioration causes off-odours, off-flavours, and texture softening when amines develop, lipids oxidise, nucleotides degrade, and proteins degrade (Alasalvar et al., 2001;Ozogul et al., 2006; Hernandez et al., 2009). Many cold water fish species, including sea bream, sea bass, sardines, and European eels, have had their freshness evaluated (Alasalvar et al., 2001; Alasalvar et al., 2002; Ozogul et al., 2004; Ozogul et al., 2006; Hernandez et al., 2009). However, few studies on the evaluation of tropical freshwater fish species quality were published (Chytiri et al., 2004).

According to a number of authors, the majority of fish and fisheries products contain microorganisms that are a reflection of the microbial community in that habitat (Gram and Huss, 1996;Adams and Moss, 2008). The total number of organisms, however, fluctuates greatly depending on a number of circumstances. The variety of processing techniques (frozen to canned products) and the wide range of environmental habitats (freshwater to saltwater, tropical waters to arctic waters, pelagic swimmers to bottom dwellers and degree of pollution) are factors that

determine the initial contamination of fish and fish products in accordance (Gram et al., 1996). The intrinsic and extrinsic parameters will decide which portion of the microflora eventually grows on fish products.The fact that fish are poikilothermic (cold-blooded) and live in an aquatic environment, a high post-mortem pH in the flesh and the existence of significant non-protein-nitrogen (NPN) content are some fundamental variables that have a significant impact on fish microbiology and spoiling .Because it determines the quality of frozen meat, thawing loss is one of the most crucial tests because it causes the meat to lose more fluid and become dry and pale. Fish lipid breakdown is the principal cause of the chemical deterioration associated with fish during storage (auto-oxidation). Fish generally has a higher percentage of unsaturated lipids than other foods. Fish lipids undergo two primary modifications, lipolysis and auto-oxidation. The primary reactants in these reactions are fish unsaturated lipids and atmospheric oxygen, which results in the synthesis of hydroperoxides, which have an unpleasant smell and cause brownish-yellow discoloration of the fish tissue. Strong rancid flavours are created when hydroperoxides continue to degrade.(Huss et al., 1997)

2.2 PROXIMATE ANALYSIS

Table.2.1. Proximate composition of five commercial fish species are tabulated below :-

Common name	Scientific name	Proximate composition				Reference
		Moisture(%)	Protein(%)	Fat(%)	Ash(%)	
1) Bayad	*Bagrus bayad*	79.10	17.22	2.23	1.03	Malik et.al.,(2021)
2) Ijeel	*Lates niloticus (L.)*	74.20	23.03	0.98	1.38	
3) Khashm el banat	*Mormyrus casahive (L.)*	72.96	19.94	6.06	1.08	
4) Bulti	*Oreochromis nilotica (L.)*	74.30	22.90	1.44	1.42	
5) Gargur	*Synodontis schall*	73.89	23.60	1.53	1.42	

Arannilewa et al.,(2006) studied the effect of frozen period on the chemical, microbiological and sensory quality of frozen tilapia fish (*Sarotherodon gallienus*) and the goal of the study was to determine how long fish were kept frozen affected their chemical, microbiological, and sensory profiles (*Sarotherodon gallienus)*. The fish were taken from an agricultural development project study pond in Akure,Nigeria and stored frozen for sixty days before being examined every ten days. The range of protein content is between 1.17% and 2.40%. Fish samples that were frozen for 60 days had the lowest protein level (43.70±1.17%), whereas fresh samples had the highest protein content (60.65±2.40%). Protein content declines with increasing time in frozen storage. Similar outcomes were found for the fat content, where

the fresh samples had the highest fat percentage (9.72±0.25%).The samples that had been held for 60 days had the lowest value, which was reported for the fresh samples. While being stored, neither the ash content nor the moisture content significantly change.These imply that fish should, if required, be refrigerated for a brief amount of time to preserve flavour and give an optimal level of both protein and fat.

Salama (2007) studied effect of freezing on the functional properties of Nile tilapia *Oreochromis niloticus* protein in which Nile tilapia fillets and minced flesh samples were periodically withdrawn and analysed for water holding capacity (WHC), foaming capacity (FC), emulsification capacity (EC), total soluble protein (TSP), soluble protein nitrogen (SPN) and soluble non protein nitrogen (SNPN) as quality criteria of Nile tilapia fillets and machine minced flesh blocks packaged in ice-glaze film or polyethylene bags were evaluated over a 6-months storage period at -20°C. Results showed that WHC, FC and EC gradually decreased in all treatments. Also TSN, SPN and SNPN were slowly decreased in all samples during the storage period. fillets blocks were much more stable than minced blocks, especially those packaged in ice-glaze film compared with those packaged in polyethylene bags.

Gupta et.al.,(2012) studied change in proximate composition by low temperature preservation in fish muscle of *Labeo rohita* in which the study was designed to investigate the effect of low temperature preservation on the chemical profile of fish muscle stored for a period of twenty one days. The proximate composition was carried out at a 7 days interval on muscle of fish during frozen storage. It was found that the protein, lipid, moisture and ash contents decreased significantly ($P < 0.05$) during the entire storage period. In fresh (unfrozen) samples, protein(15.93±0.04%), fat (3.86±0.04%), moisture (84.74±0.1%.),and ash content (1.79±0.01%) were found to be the highest ,where as the total percent decrease in frozen samples on 21st day of frozen storage was (12.99%), (22.27%) , (4.60%) and (24.02%) for protein, fat, moisture and ash respectively.

Khidhir et al., (2013) studied qualitative assessment of imported frozen fish fillets in Sulaimani markets in which the study aimed to determine the quality of frozen fish fillets sold in Sulaimani city markets. A total number of (64) samples of frozen fish fillets belonging to 4 foreign trademarks were collected from different parts of Sulaimani markets. The samples were subjected to physical tests to determine their quality and suitability for human consumption. The proximate chemical analysis referred to the presence of significant differences in moisture, fat and protein content among the four trademarks except for ash content. While the physical indices showed that White fish fillets recorded the lowest thawing and cooking loss which in return recorded the highest WHC.

Subbaiah et al., (2015) studied protein degradation and instrumental textural changes in fresh nile tilapia (*Oreochromis niloticus*).During frozen storage looking at the texture changes and protein degradation that occurred following 150 days of frozen storage of Nile tilapia (*Oreochromis niloticus*). During the frozen storage period, salt-soluble protein and protein

solubility considerably decreased. After performing sodium dodecyl sulphate polyacrylamide gel electrophoresis, it was discovered that the thickness of the band had decreased but that there had been no discernible alterations in the myosin heavy chain up until the 90th day of frozen storage. On the 120th and 150th day of the sampling period, a new lighter band with a molecular weight of 29 kDa was discovered. More proteolysis resistance was discovered for actin.Analysis of the texture profile showed that up to the 120th day, gumminess, chewiness, and hardness all steadily declined, but that they then increased.Even though sensory qualities started to deteriorate as the frozen storage period went on, the fish was still in acceptable condition after 150 days.The sensory panelists noted a gradual hardening of the muscle.

El-Lahamy et al.,(2019) studied effect of frozen storage periods and frying process on chemical composition of the Nile tilapia fish (*Oreochromis niloticus*) in which changes in the tilapia fish's (*Oreochromis niloticus*) moisture, protein, fat, and ash contents following freezing at -18°C for six months and their pre-frozen fried products were assessed. In June 2016 at the Fayoum governorate in Egypt, fish samples were collected from two resources: (A) farms watered by el-bats discharge and (B) farms irrigated by el-wadi discharge. According to the findings, the raw tilapia (A) and (B) samples had moisture contents of 79.41% and 79.38%, crude protein contents of 18.05% and 17.89%, lipid contents of 1.06 and 1.07%, and ash contents of 1.14 and 1.22%, respectively. Based on changes in moisture content that happened over long periods of storage, all of these values changed. On the other hand, it was shown that protein, fat, and moisture content values dropped as All fried samples had rising ash concentrations during the course of storage. In conclusion, even when the resources used to irrigate fish farms varied, the chemical makeup of fish remained consistent. The amount of time the food was stored and how it was fried both had an impact on changes in chemical composition. The nutritional value of tilapia fish was preserved, nonetheless, up until the conclusion of the frozen storage period.

2.3 PHYSICAL ANALYSIS

Purwaamidjaja et al.,(2010) studied storage quality of fresh redfish (*Sebastes marinus*) fillets as affected by different cooling methods in which the impact of pre-cooling previous to packaging via way of means of the usage of combos of slurry ice immersion and integrate blast and contact (CBC) cooling technology on physicochemical houses of sparkling redfish *(Sebastes marinus*) fillets throughout processing and garage at -2°C to 2°C was investigated. The cooling remedies did now no longer affect the water content material and lipid content material of the redfish fillets. Meanwhile, the growth in drip and the cooking yield throughout the garage could be due to decomposition of the fish muscle. CBC cooling improved the water holding capacity (WHC) of the fillets. Slurry ice immersion of the redfish fillets brought about improved lipid oxidation, even as CBC cooling bogged down lipid oxidation.

Gang (2013) reported changes in the quality and yield of fish fillets due to temperature fluctuations during processing in which the goal was to map the ambient and fish fillets

temperature in a fish processing plant. Furthermore, to analyse the consequences of ambient temperature (10, 16, 22°C) and conserving time (0, 0.5 , 1.0, 1.5, 2.0, 2.5 hours) on temperature rises and drip loss in fresh redfish and saithe fillets. During next chilled storage (2±2°C), drip and pleasant changes of saithe fillets that have been stored at 16±2°C for 0, 1, 2 hours earlier than packaging have been studied. Results confirmed that the very best ambient and fillet temperature seemed in packaging regions of the processing plant. Longer conserving time and better ambient temperature in the course of processing led to massive drip losses and temperature rises within the fillets.

Zhi-qiang et al.,(2017) studied the effect of physical osmosis methods evidently, the samples with ultrasonic assisted osmotic pretreatment showed less drying time and more aromatic substances whereas the samples from the periodic vacuum assisted osmotic pretreatment had higher supermolecule protection feature. Though the dried samples had higher quantitative relation of certain water and better storage stability when these 2 pretreatment methods, from the purpose of reading of skyrocketing drying rate and stimulating flavour substances, the ultrasonic assisted osmosis pretreatment methodology had a lot of advantages. The analysis outcomes will contribute to optimising higher pretreatment ways for the method of warmth pump dried tilapia fillets. quality of tilapia fillets processed by heat pump drying so on the way to reap the effect of various pretreatment methods on warmth pump dried tilapia fillets, the outcomes of trehalose, ultrasound-assisted and freeze-thaw cycle assisted osmotic dehydration on the coloration, rehydration, texture and Ca2+-ATPase pastime had been investigated. Tilapia fillets (100 mm period × 50 mm width × 5 mm height) had been first osmo concentrated in a trehalose solution mixed with four°C beneath atmospheric pressure for 1 h, different power of ultrasound and freeze-thawing respectively, then heat pump drying. The results confirmed that under the same drying method, the complete score of ultrasound in four hundred compared to freeze-thaw, the ultrasound pretreatment had a large ($P<0.05$) effect on the colour and Ca2+-ATPase activity, but had no significant ($P>0.05$) effect on the rehydration and texture. But, each of them notably ($P< 0.05$) affected the first-class in evaluation to that of osmosis at 4°C. It shows that appropriate ultrasonic pretreatment conditions enhance the high-quality of dried merchandise successfully and the belief of these studies gives reference for heat pump dried comparable products.

Giannakouros et al.,(2019) studied shelf life extension and improvement of the nutritional value of fish fillets through osmotic treatment based on the sustainable use of rosa damascena distillation by-products in which the objective of this work was comparative study of various diffusion treatments at 37◦C on the standard and period of chilled ocean bass fillets. Fish fillets were treated mistreatment osmotic solutions consisting of oligofructose (40%–50%–60%) and 5% NaCl with (BP/OT) and while not (OT) former inhibitor enrichment by using damask rose distillation by-products. Water activity diminished to more or less 0.95 once 330 minutes of osmotic treatment. Untreated and osmotically treated fish fillets (BP/OT) and (OT) were

afterwards hold on at 5◦C and their quality was evaluated primarily based on microorganism growth and supermolecule oxidation. Osmotic treatment extended considerably the period of fish in terms of microorganism growth; however, it conjointly accelerated its supermolecule oxidation. The impregnation of damask rose phenolics not solely counterpoised this negative effect, however diode to a over four-fold increase of the shelf life of ocean bass, as compared to the untreated samples.

Li et al.,(2019) studied effect of pretreatment on water migration and volatile components of heat pump dried tilapia fillets.In this study, unhearable motor-assisted diffusion pretreatment and periodic vacuum assisted osmotic pretreatment were applied to research their effects on water migration and volatile elements of warmth pump dried tilapia fillets. To realise that, some effective parameters together with sample drying rate, water diffusivity, microstructure, water morphology, water distribution, and volatile components were compared and analysed with some advanced activity devices. The water diffusivity, water distribution characteristics, and composition of volatile components were obtained when totally different pretreatment methods. Because the drying method progresses, the sample wetness content decreases. Meanwhile, the high-degree-of-freedom water migrates to the low-degree-of-freedom water and therefore the water-solid bond strength increases. Subsequently, the effective water diffusion coefficients of management cluster (without pretreatment samples), unhearable motor-assisted diffusion pretreatment group and periodic vacuum assisted osmosis pretreatment group were measured as 4.304×10^{-7}m/s, 6.109×10^{-7}m/s, and 5.003×10^{-7}m/s, respectively. In addition, the control group, ultrasonic assisted osmosis group, and pulse vacuum assisted osmosis group contained 52, 59, and 41 volatile compounds, respectively. Compared to the results from the control group, the water diffusion coefficients of ultrasonic diffusion pretreatment and pulse vacuum osmotic pretreatment exaggerated by 41.94% and 16.24%, respectively. From the purpose of reading the skyrocketing drying rate, the ultrasonic penetration pretreatment provided higher improvement, which was precisely in keeping with the results of microstructure. On the opposite hand, the unhearable motor-assisted diffusion pretreatment cluster had a lot of varieties of volatile compounds that may stimulate more flavoured substances to be released.

2.4 BIOCHEMICAL ANALYSIS

Aubourg et al., (2004) studied quality loss related to rancidity development during horse mackerel (*Trachurus trachurus*) frozen storage.The development of rancidity and its effect on quality loss was studied in frozen horse mackerel (*Trachurus trachurus*). For that, two different kinds of fish products (whole fish and fillets) were stored at a commercial frozen temperature (-20ºC) for up to 12 months and were compared to samples stored at a much lower temperature (-80ºC). According to biochemical indices, fillets stored at –20ºC showed susceptibility to rancidity development, leading to a shelf life of 1 month, while whole fish at the same temperature were still edible at month 5. The employment of a low temperature (-80ºC) inhibited

the rancidity development leading to good quality (whole fish) and fair quality (fillets) fish products at the end of the experiment. The application of protective treatments specially designed to prevent lipid oxidation is encouraged when commercialising this species in the frozen state.

Aubourg et al.,(2007) studied development of lipid changes related to quality loss during the frozen storage of farmed coho salmon (*Oncorhynchus kisutch*) in which lipid changes related to quality loss were evaluated during frozen storage of coho salmon for up to 15 months. Biochemical indices concerning lipid hydrolysis (free fatty acids, FFA) and oxidation (peroxide value, PV; thiobarbituric acid index, TBA-i; fluorescent compounds, FR; polyene index, PI) were determined .As a result of the frozen storage, lipid hydrolysis was shown to develop according to the increase in FFA content ($p < 0.05$). However, most biochemical lipid oxidation indices (PV, TBA-i and FR) led to a low degree of rancidity development ($p < 0.05$) when compared to other fatty fish species under similar frozen storage conditions. The PI value decreased ($p < 0.05$) at month 10 but then remained unchanged until the end of the experiment. Rancid odour and taste development were shown to be low throughout the experiment, according to the biochemical indices mentioned above. However, a progressive decrease ($p < 0.05$) in the original fresh odour and taste of salmon fish flesh occurred with increasing frozen storage time, such that fish samples had the poorest scores by month 15. Endogenous antioxidants were remarkably stable throughout the experiment and which might contribute to the oxidative stability of frozen farmed coho salmon lipids.

Ozogul et.al.,(2009) studied biochemical assessment of marinated anchovy fillets stored at 1±1°c in which biochemical (TVB-N, TBA, peroxide price, free fatty acids, biogenic amines, and pH) parameters of marinated anchovy fillets hold on at 1±1 °C were evaluated. The concentrations of TBA and PV were found to be a lot more economical than TVB-N within the determination of quality of marinated anchovy fillets. The initial PV value was found to be 1.48±0.45 meq/kg and reached the highest level of 25.83±2.19 meq/kg. The histamine level enhanced throughout the storage amount associated reached 3.45±2.00 mg/100 g however didn't exceed the level by the food and drug administration (FDA). Knowledge obtained from this study shows that marinated fish samples are often held on for seven months at 1±1 °C.

Khidhir et al.,(2013) studied qualitative assessment of imported frozen fish fillets in Sulaimani markets in which the study aimed to determine the quality of frozen fish fillets sold in Sulaimani city markets. A total number of (64) samples of frozen fish fillets belonging to 4 foreign trademarks were collected from different parts of sulaimani markets.The samples were subjected to quality and suitability for human consumption. Chemical indices showed that the pH mean values of myanmar and flander mark were significantly differed ($P<0.05$) than Hasson and white fish fillet, Although, the results of FFA recorded no significant differences among the trademarks, and flander mark recorded the highest PV and TBA among the other which made it significantly differed than them and White fish fillet recorded the lowest, still, they were within

the international standard limits. Where, the results of TVN values recorded no significant differences among the inspected marks. All obtained results referred to the validity of these fish fillets for human consumption.

Karami et al.,(2013) studied the effects of frozen storage on biochemical quality indices of red tilapia (*Oreochromis niloticus* × *Tilapia mossambicus*) fillets and the investigation's goal was to ascertain biochemical quality indicators of red tilapia fillets changed when they were frozen and stored at -18°C. The fish were manually filleted. After that, the prepared fillets were put into the polyamide bags and kept at -18°C for 150 days. For a five-month period, the biochemical quality indices were determined. According to the findings, 29 fatty acids were found in both the fresh and frozen samples. The thiobarbituric acid value (TBA.Mg malondialdehyde/kg) considerably increased (p0.05) from 0.03 to 1.26 during storage. Although the peroxide value (PV), total volatile bases (TVB-N), and pH value were all still well below acceptable limits.

Cárdenas et al., (2015) studied freezing and freezing-thawing cycles on biochemical changes of meagre (*Argyrosomu regius*) fillets during further cold storage in which the results of freezing and freezing-thawing cycles at some stage in bloodless garage were studied in meagre (*Argyrosomus regius)* fillets. Fillets have been subjected to 3 conservation protocols: clean, freezing at –20◦C, and repeated freezing-thawing cycles. Fresh fillets have been stored (4◦C, 15 days), and the same protocol became observed for freezing and freezing-thawing after the freezing period. Freezing and freezing-thawing fillets have been softer and provided decreased water conserving ability than clean, in the main attributable to collagen solubilization, and partial myofibrillar protein degradation. Cold garage (4◦C) at some stage in 15 days precipitated softening in clean and frozen fillets as a result of myofibrillar protein hydrolysis. Freezing-thawing cycles elevated proteolysis, this led to unacceptable softening even from early tiers of similarly bloodless garage, and this became additionally discovered via means of SDS-PAGE.

Khajehrahimi et al., (2018) studied effect of different freezing processes on the quality and histological changes of red tilapia (*Oreochromis niloticus* × *Tilapia mossambicus*)**.**The fillets were packaged, frozen using both too-slow and too-quick procedures, and kept at -18°C for six months. Then, monthly evaluations of fillet alterations, including total volatile basic nitrogen (TVB-N), peroxide value (PV), and thiobarbituric acid value (TBA), were conducted. Results showed both quick and slow freezing produced changes in all examined attributes over the course of various months, though slow freezing produced changes that were more pronounced. Greater fluctuations were seen with slow freezing for the PV (0.02-0.93 mEq kgG1), TBA (0.03-1.2 mEq kgG1), and TVB-N (12.63-21.93 mg/100 g) values.On the other hand, it found that the freezing approach caused the fillets to degrade less. Conclusion was that when compared to samples frozen slowly, TVB-N, PV, TBA were less pronounced in samples frozen quickly.

Zhao et al.,(2019) studied effect of vacuum impregnated fish gelatin and grape seed extract on metabolite profiles of tilapia (*Oreochromis niloticus)* fillets during storage in which the objective of this study was to investigate the effect of vacuum impregnated fish gelatin (FG) and grape seed extract (GSE) on metabolites of tilapia fillets during storage using nuclear magnetic resonance (NMR). Totally 42 metabolites were identified, 36 of which were quantified. The multivariate analysis results demonstrated distinct separations between fresh and stored fillets, indicating significant metabolite changes during storage. Some metabolites like choline and trimethylamine oxide were closely related to freshness while organic acids were associated with spoilage. Combined FG and GSE reduced the formation of undesirable metabolites like trimethylamine and histidine significantly ($P < 0.05$). Traditional freshness indexes indicated preserved quality after combined coating and further verified NMR results. This study reveals the potential of NMR to analyse metabolites that determine fish quality and to monitor their changes during storage.

Ibrahim et al.,(2019) reported changes in biochemical criteria of tilapia fish samples during frozen storage at -18℃ for 180 days and their fried products in which tilapia samples were stored frozen for 180 days at -18°C, and changes in the biochemical criteria of the fish's fried products were examined. For farms A and B samples, the quality standards for the raw samples were 6.74 and 6.2 pH; 10.64 and 12.04 mg/100 gm TVB-N; and 0.21 and 0.23 mg/kg TBA, respectively. TBA, TVB-N, and pH levels all increased after frying in fried samples made from frozen samples stored for various amounts of time. While pH values of frozen samples declined to 6.61 in farm A frozen samples, on the other hand, pH values were increased to 6.53 in samples of farm A after 60 days of freezing, TVB-N and TBA values increased with storage periods till the end of storage. Following that, pH readings climbed until the end of storage.

Tenyang et al.,(2019) studied alteration of the lipid of red carp (*Cyprinus carpio*) during frozen storage.The aim of this study was to work out the aerophilic stability of oil extracted from red carp fish frozen up to 9 months at −18°C. To assess oil stability of red carp fish, the analytical indexes and Fourier remodel infrared (FTIR) spectrometry were used. These methodologies used provided similar conclusions. Before frozen storage, the composition of fatty acids showed that red carp oil could be a sensible supply of unsaturated fatty acids (PUFAs) resembling polyunsaturated fatty acid (C18:2ω-6: 5.29% of total fatty acid), omega-6 fatty acid (C18:3ω3: 3.53% of total fatty acid), arachidonic acid (C20:4ω6: 3.68% of total fatty acid), omega-3 (C20:5ω-3, EPA: 4,06% of total fatty acid), and omega-3 (C22:6ω-3: 3.02% of total fatty acid). throughout frozen storage, the free fatty acid and peroxide worth increased, respectively, from 1.35% to 8.06% in monounsaturated fatty acid and 3.77 to 18.62 meq O_2/kg in lipid, whereas the magnitude relation of PUFA/SFA and polyene index attenuated, respectively, from 0.58 to 0.25 and 0.30 to 0.09. The triglycerides conjointly decreased with frozen duration. Therefore, for good fish quality, red carp fish should be keep for<3 months at −18°C.

Ruan et al.,(2022) studied the vacuum impregnation process optimization for tilapia with biopreservatives at ice temperature in which the vacuum impregnation (VI) method was accustomed pretreat fish genus fillets with biopreservatives at −2◦C. Response surface methodology (RSM) was utilised to optimise process conditions, as well as vacuum pressure (pv), vacuum maintenance time (t1), and air pressure recovery time (t2), that were determined to be 67.73 kPa, 23.66 min, and 8.87 min, respectively. The anticipated values for (TVB-N) were 14.04 mg/100 g, Verification experiments were conducted, and therefore the experimental results of TVB-N deviated 0.64%. After thirty days of storage following VI and atmosphere impregnation (AI) pretreatment TVB-N was determined. On the thirtieth day, the results for VI pretreatment was 17.41 mg/100 g and TVB-N ablated by 29.6%. This study demonstrates that when biopreservatives are applied throughout the pretreatment process, VI technology is often utilised to facilitate their penetration into the interior of tilapia, thus considerably enhancing the impact of ice-temperature preservation.

2.5 MICROBIOLOGICAL ANALYSIS

Odoli et.al.,(2009) studied optimal storage conditions for fresh farmed tilapia (*Oreochromis niloticus*) fillets. The major objective was to define the best conditions for storing fresh tilapia fillets by calculating their shelf life based on microbiological evaluations In order to do this, Nile tilapia (*Oreochromis niloticus*) raised in a recirculation aquaculture system was filleted and packaged in 100% air and 50% CO_2: 50% N_2 MA before being stored at different temperatures; 1°C and -1°C. For comparison, initial filleting samples (control d0) were also assessed. The development of a Quality Index Method (QIM) scheme is also covered.For all sample groups tested in the main study, the QIM scheme's application to tilapia fillets revealed a linear relationship between QIM scores and storage period with strong correlations ($r > 0.93$). According to the results of microbiological growth, fillets packaged in 100% air had a shelf life of 13–15 days at 1°C and 20 days at -1 °C. TVC and pseudomonas counts in flesh at the end of shelf life in 100% air packaged groups reached $\log_7$ CFU/g.The lag phase and development period of bacteria were prolonged in MA packed fillets, and counts below the allowable consumption level ($\log_4$ CFU/g) were observed up to 27 days of storage at both 1°C and -1°C.

Liu et al., (2010) studied quality evaluation of tray-packed tilapia fillets stored at 0°C based on microbiological attributes in order to explain the mechanism of fish spoiling and create the most trustworthy indicators for microbiological assessments of tray-packed tilapia genetically improved farmed tilapia strain of (*Oreochromis niloticus*) fillets were researched. The findings demonstrated when the microbiological load was greater than $\log_6$cfu/g, protein breakdown could be seen on the SDS-PAGE. A extremely low value for thiobarbituric acid reactive substances (TBARS) persisted over the course of storage, indicating minimal lipid oxidation in muscle.The shelf life of tilapia fillets held at 0°C was roughly 10–12 days, taking into account fish freshness and microbiological safety.

Gupta et.al.,(2012) studied change in microbial count by low temperature preservation in fish muscle of *labeo rohita*(hambuch) in which the study was designed to investigate the effect of low temperature preservation on the microbial profile of fish muscle *(labeo rohita*) stored for a period of twenty one days. The microbiological analyses were carried out at a 7 days interval on muscle of fish during frozen storage.The microbial count increased gradually during the period of storage. The total plate count in fresh fish muscle on day zero was rather low i.e. 2.44±0.2 log cfu/g as compared to the values found on the 21st day i.e. 5.10±0.02 log cfu/g. The Coliform Count also followed the same trend during the storage. On day zero, a few colonies were found and the CC was 1.50±0.15 log cfu/g whereas it increased up to 3.08±0.07 log cfu/g on the last day of storage. Similarly Psychrophilic count increased from 2.15±0.2 log cfu/g on day 0 to 5.06±0.05 log cfu/g on 21st day. Thus, a significant quality loss was observed in fish during storage. However, the present frozen conditions retained the fish under acceptable microbial conditions for human consumption up to 14th day beyond which it became unfit for human consumption

Johannsson et al.,(2013) studied shelf life of air and modified atmosphere-packaged fresh tilapia (*Oreochromis niloticus*) fillets stored under chilled and superchilled conditions By assessing microbiological changes, the best packaging and storage conditions for fresh tilapia fillets were found. Prior to chilling and superchilling storage at 1°C and 1°C, Nile tilapia *(Oreochromis niloticus*) raised in recirculation aquaculture systems was filleted, deskinned, and packaged in air and 50% CO_2/50% N_2. Cooked sample analysis showed that air-packaged fillets had a shelf life of 13–15 days at 1°C and 20 days there. Total viable counts (TVC) and pseudomonads counts reached $\log_8$ colony-forming units (CFU) g1 at the end of the shelf life in air-packaged fillets.In 50% CO_2 after 23 days of storage at both 1°C and 1°C, N_2-packaged fillets' recorded counts were below the limit for ingestion ($\log_8$ CFU g1) and the lag phase and production time of bacteria were lengthened.

Sajjan et.al.,(2014) studied the microbiological characteristics of mechanically deboned tilapia fish [*Oreochromis mossambicus*] meat under frozen condition in which the study was undertaken to investigate the influence of frozen condition on characteristics of deboned tilapia fish meat. The microbiological analyses were carried out at 1, 3, 5, 7, 14, 28, 60 and 90 days during frozen storage.. The total plate count in fresh deboned fish meat samples reduced from 2.51×10^6 to 0.98×10^6 cfu/g after 90 days of storage. The lactic acid bacteria decreased from 9.8×10^4 to 6.4×10^4 cfu/g and the H_2S- producing bacteria reduced from 9.9×10^4 to 7.2×10^4 cfu/g after 90 days of frozen storage. The E. coli bacteria were not detected throughout the storage. period. However, the frozen stored tilapia fish meat samples retained their quality acceptable for human consumption after 90 days of storage.

Surendra et al.,(2018) studied effect of low-dose gamma irradiation on the quality of tilapia fish muscle with storage at 0°C in which the impact of gamma irradiation, accompanied by way of means of garage in ice, on tilapia (*Oreochromis sp.)* fillets changed into investigated

via way of means of tracking microbiological and chemical adjustments after low-dose irradiation (1 and three kGy). Control and irradiated samples have been saved in ice, and have been analysed at 7-day intervals. Bacterial counts confirmed that the shelf-existence of three kGy irradiated tilapia prolonged to 70 days, and 1 kGy irradiated tilapia had a shelf-existence of 56 days. By comparison, managed samples had a shelf-existence of 7 days

CHAPTER III : MATERIAL AND METHODS

3.1 Materials :

3.1.1 Tilapia :

Freshwater tilapia (*Oreochromis niloticus)* locally known as chilapi were cut into fillets and were used for storage study.

3.1.2 Chemicals :

Sodium tripolyphosphate

Commercially available sodium tripolyphosphate from M/S.AlbrightMoraji and Pandit Ltd. Mumbai used for pretreatment.

Sodium Chloride.

Salt was used for pretreatment

Culture media :

All the chemicals and media used in the study were either of bacteriological or analytical grades. All the media and chemicals used in this study were stocked in the laboratory at Postgraduate Institute Of Post Harvest Management, Killa-Roha, Raigad.

3.1.3 Glass wares:

All glass wares used for analysis study were procured from Borosil Laboratories, India

3.1.4 Packaging material:

High density polyethylene (HDPE) bags were used for packing.

3.1.5 Equipment, machineries and utensils:

3.1.5.1 Weighing balance:

Electronic Monopan balance of 'Milton' make (Citizen Scale Pvt. Ltd. Mumbai, India) was used for weighing purposes.

3.1.5.2 Water bath:

A water bath of 'Bio-Technics' was used for analytical purposes.

3.1.5.3 Refrigerator:

A 'Vestfrost' made refrigerator was used for storage.

3.1.5.4 Autoclave:

Autoclave of 'Equitron' brand (Medical Instruments mfg. Co, Mumbai) was used for sterilization of glassware and media.

3.1.5.5 Hot air oven:

A hot air oven (Nishitronics instruments, Pune) was used for drying and moisture estimation.

3.1.5.6 Muffle furnace:

Muffle furnace (Classic scientific, Mumbai) was used for estimation of ash.

3.1.5.7 Incubator:

Bacteriological 'Yorco' brand incubator was used for incubation of samples in petri dishes with media.

3.1.5.8 Colour reader:

Konica Minolta colour Reader was used for fish colour analysing.

3.1.5.9 Texture analyser:

TA XT2i texture analyser was used for fish texture analysing.

3.2 Methods:

3.2.1 Preparation of raw materials:

Tilapia was procured from the market and filleted. The fillets were taken to the factory in an icebox. The fillets were then washed thoroughly and divided into 15 each for 4 different treatments as T0,T1,T2,T3. Firstly the untreated/control (T0) were frozen by IQF. Then the remaining fillets were treated with 2% Nacl and 3 % STPP for 2 hrs.T1 & T2 are glazed & blanched as given below and only T3 fillets were used for blast freezing, remaining T1,T2 fillets were separately IQF.At last all fillets were put into separate LDPE packets according to their treatments and packed in a corrugated fibre board & kept in frozen storage at -18°C.The flow chart of the process is as follows:-

3

Raw material (fillets) arrival (In iced condition)

Filleting

Fillets are washed with water

Wash and chill room

1.(Untreated/Control T0)

2. Treatment (Nacl+ sodium tripolyphosphate)

IQF Freezing (T1,T2,T2)

Air blast freezing (T3)

Weighing

Packaging and labelling

(Primary Packaging material: Monolayered

LDPE or LLDPE linear low density polyethylene)

(Secondary Packaging Material : 3 – 5 ply Corrugated fire board)

Frozen storage at -18 °C for 4 months

Different treatments used for fillets were :-

T0 : Control/Untreated IQF at freezing temp - (- 40 ˚C) & storage at (-18 ˚C) for 120 days.

T1 : Pre-treatment of NaCl (2%)+ STPP (3%) for 2 hrs + glazing - 10% + IQF freezing (Temp: - 40˚C) & frozen storage at (-18˚C) for 120 days.

T2 : Pre-treatment of Nacl (2%)+ STPP (3%) for 2 hrs + blanching at temp:75˚c, Time: 2 Min + glazing - 10% + IQF freezing (temp: - 40˚C) & frozen storage at (-18˚C) for 120 days.

T3 : Pre-treatment of NaCl (2%)+ STPP (3%) for 2 hrs, blast freezing & frozen storage at (-18˚C) for 120 days.

3.2.2 Storage studies:

The sample was stored at a refrigerated storage temperature of -18°C for a period of four months. The stored sample was analysed for different proximate, biochemical, microbiological quality and sensory parameters at 0 day (day of storage) and regular interval of every 15 days from the day of storage.

3.2.3 Sampling:

For refrigerated storage studies, samples were drawn randomly at every 15 days interval to analyse the changes in proximate, biochemical, microbial and sensory parameters. The samples were packed individually in HDPE bags to store separately for sampling. A single bag of packed samples was taken for analysis without disturbing other sample bags at the time of sampling, and sampling was done with that sample.

3.3 Analysis:

The samples stored at a refrigeration storage temperature were analysed for the proximate composition, biochemical, microbial and sensory quality characteristics. Moisture, crude protein, crude fat, ash content,and total volatile bases nitrogen (TVB-N) were analysed. The microbiological quality parameter were analysed in duplicate i.e. total plate count (TPC). Whereas sensory scores are the mean values as assessed by 10 semi trained panellist on numerical scores on 9 point hedonic scale.

3.3.1 Proximate composition

Proximate composition was analysed by the method described in AOAC (2005).

3.3.1.1 Moisture

The determination of moisture content was carried out by (AOAC, 2005). A sample (10 g) was weighed into a previously weighed petri dish and placed in a hot air oven at 105°C ± 20°C for 16 - 18 hr. The petri dish was cooled and kept in desiccators and the weight of the petri dish was noted. The process of heating, cooling and weighing was

repeated until the difference between two successive weights was less than one mg. The lowest weight was recorded. Then moisture content was expressed as percentage by weight using the following formula

Moisture% = W_2-W_1 / W_0 X 100

Where,

W= Weight (gm.) of test sample

W= Weight (gm.) of petri dish + test sample

W= weight (gm.) of petri dish + test sample after drying

3.3.1.2 Protein

Protein content was estimated by Kjeldahl's method according to AOAC (2005).

Digestion:

A sample (1 g) was taken and transferred to Kjeldahl's digestion flask. 0.5 g of digestion mixture (K_2SO_4: $CuSO_4$: 5:1) was added to it. Then 20 ml concentrated sulphuric acid was added and mixed. The solution was heated until it became clear.

Distillation:

The digested sample was transferred to a 250 ml volumetric flask and volume was made up with distilled water. 10 ml of sample was taken with a volumetric pipette and poured into a distillation unit. 10 ml of 40% NaOH solution was added. The top of the funnel was closed and filled partially with distilled water. 10 ml Boric acid (2%) solution was taken into a 100 ml conical flask. Steam was generated by boiling the water. The sample was distilled for about 15 min after bubbling. After collection of 30 - 40 ml of distillate, boric acid turns from blue violet to green colour. Distillate was titrated with 0.143 N H_2SO_4 solutions until blue violet end point persists.

Total nitrogen%=1.4 X (ml HCL-ml blank) x Conc.of HCL/ Weight of sample(g) X 100

% Protein = % total nitrogen x 6.2

Where,

N = Normality of H2SO4

V = ml of digested extract taken for distillation

W = weight (g) of sample

3.3.1.3 Fat

Fat content of was estimated according to AOAC (2005). The dried sample (2gm) was weighed on filter paper and placed into a thimble. The thimble was placed in an

extraction tube, which was then connected with the weighed flask containing sufficient ether and also with the condenser. The apparatus was heated to 55 – 60ºC. The heat vaporised the volatile solvent, which passed up through the side arm and was condensed in a condenser. The condensed solvent fell drop by drop into the porous thimble. When sufficient solvent has been transferred to the extraction tube to fill the syphon arm, it syphons back over into the weighted flask. This process was continued until the extraction was completed in 8 - 10 hrs. The flask was removed and the volatile solvent was evaporated.The flask with content was expressed in percentage and was calculated by formula

Fat%= W_2-W_1/ W X 100

Where,

W = Weight of sample in gm

W1= Initial weight of beaker in gm

W2= Final weight of beaker in gm

3.3.1.4 Ash

Determination of ash content in dried was carried out by (AOAC, 2005). The sample (2 gm) was weighed accurately in a porcelain crucible. Flame of a burner was ignited for about one hour to smoke off,excess moisture, fat etc.; and then transferred to the muffle furnace and heated at 550°C for 5 hr. Then it was directly transferred to desiccators, cooled and weighted. Ash content was analysed using the following formula :-

Ash%= W1-W2 / W1-W X 100

Where,

W= Weight (gm) of empty crucible

W= Weight (gm) of crucible + dried sample

W= Lowest weight (gm) of crucible with sample

3.3.2.1 Physical parameters :

Drip loss :

Take a sample day 0 were individually weighed and recorded as initial weight(W1).The samples were then placed in sealed polyethylene plastic bags,vacuum-packaged,placed within a container and were stored in a chiller at 4 degrees.After 1 and 7 d of storage,the samples were immediately removed from bags,gently blotted dry,weighed and recorded as W2 (final weight).The percentage of drip loss was calculated and expressed as the percentage of differences of sample initial weight. The sample weight after 1 and 7d of storage was divided by sample initial weight (Honikel 1998) using the following equation;

Drip loss (%) = [(initial weight − weight after thawing)/initial weight]×100

3.3.2.2 Colour:

Konica minolta colour reader was used.The Color readings were expressed by machine (L*a*b*) system (Marcet et al.,2018).L*,a* and b* indicate the whiteness/darkness,which could be white. The minimum for L* would be zero,which could be black.The axes have no numerical limits.Positive a* is red and negative of a* is green.Positive of b* is yellow and negative of b* is blue.The Color of the samples was evaluated after 10 min cooling at room temperature.

3.3.2.3 Biochemical analysis

3.3.2.4 Determination of total volatile base nitrogen (TVB-N):

TVB-N content was determined by the procedure given by Beatty and Gibbons (1937) using Conway micro diffusion units and results were expressed in terms of nitrogen mg/100g.

Preparation of TVB-N extract:

5g of blended fish muscle is taken and ground in a mortar with anhydrous sodium thiosulphate and 10 ml of TVB-N reagent is added. The mixture is ground well. It is filtered and residue is washed with distilled water containing a few drops of TVB-N. This protein free filtrate is made up to 100 ml. Conway cups having a double chambered round units in which inner circular chamber is shallow as compared to outer circular chamber, and lids are washed and dried. Paraffin wax and Vaseline in the ratio of 1:2 is melted and cooled. This is applied on the rims of cups. 1 ml of N/100 H_2SO_4 is added into the inner chamber of each cup. Lid is placed over the Conway cup covering part of the outer chamber and complete inner chamber. 1 ml of TVB-N extract is kept in the outer chamber followed by 0.5 ml of HCHO. The unit is rotated to ensure mixing and then 1 ml of K_2CO_3 is added to the outer chamber. Contents are mixed by rotating the unit gently and then the unit is left overnight for reaction (it can be kept inside an incubator at 36ºC for 2h). The excess acid left in the inner chamber is titrated against N/100 NaOH using a drop of Tashiro's indicator. A reagent blank is run simultaneously

1 ml of 0.01NH_2SO_4=0.14 mg of TVB-N

3.3.3.5 Microbiological quality:

Samples were analysed for total plate count(TPC).

3.3.3.5.1 Enumeration of Total plate count (TPC):

Total plate count was determined as per APHA, 2001 and expressed as colony forming unit/g(cfu/g). Samples were tested for total plate count; physiological saline (0.85%) was used as diluent for preparation of homogenate; 25 gm sample was aseptically weighed and transferred to 225 ml of physiological saline in homogenizer. Samples were homogenised

using mortar and pestle. Appropriate dilution was prepared from homogenate using physiological saline and plated on plate count agar by pour plate method as per (Collins, 1995).The petri dishes containing sample were incubated at 37°C for 24 –28 hrs. The colonies developed on agar plates were counted and calculated. Dilution giving colonies between 30-300 ranges were selected. Plates with crowded or spread colonies, which could not be counted, were discarded. The TPC was found as under:-

Total Plate Count/g = Avg. of count in duplicate plates × dilution factor / Weight of sample

3.3.3.5.2 Sensory evaluation

Table.3.1. Score card for sensory evaluation

Organoleptic Score	**Rating**
9	Like extremely
8	Like very much
7	Like moderately
6	Like slightly
5	Neither like nor dislike
4	Dislike slightly
3	Dislike moderately
2	Dislike very much
1	Dislike extremely

Samples were subjected to sensory evaluation after brining. The fillets were kept out of the packet at room temperature for half an hour and then used for brining for sensory evaluation. Carried out for 5 minutes and allowed to cool down and then give it to the panel for sensory analysis.10 members panel composed of students and faculty conducted sensory evaluation. Each panellist was asked to evaluate the characteristics like Appearance, colour, texture, odour, flavour, taste and overall acceptability of each sample on 9–point hedonic scale (Ranganna, 1986).1 very poor and 9 excellent.

3.3.3.5.3 Statistical Evaluation

The data were analysed to test significant differences by applying an analysis of variances (ANOVA) tool available in Ms-Excel 2010. The significant differences were tested by 5% level of significance and are mentioned as $p<0.05$ for significant differences (Panse and Sukhatme,1989).

CHAPTER lV: RESULTS AND DISCUSSION

The present study entitled, "**Freezing and Storage performance of Tilapia Fillets.**" was undertaken in the Department of Post-Harvest Management of Meat,Poultry,Fish during the year 2021-2022. The experiment consisted of four different treatments on fillets.

T0 : Control/Untreated IQF at freezing temp - (- 40 ˚C) & storage at (-18 ˚C) for 120 days.

T1 : Pre-treatment of NaCl (2%)+ STPP (3%) for 2 hrs + glazing - 10% + IQF freezing (Temp: - 40˚C) & frozen storage at (-18˚C) for 120 days.

T2 : Pre-treatment of NaCl (2%)+ STPP (3%) for 2 hrs + blanching at temp:75˚c, Time: 2 Min + glazing - 10% + IQF freezing (temp: - 40˚C) & frozen storage at (-18˚C) for 120 days.

T3 : Pre-treatment of NaCl (2%)+ STPP (3%) for 2 hrs, blast freezing & frozen storage at (-18˚C) for 120 days.

The experiment consisted of four different treatments on fillets.The experimental data was analysed statistically using Factorial Completely Randomised Design (FCRD). The observations of proximate,physical,bio-chemical,microbiological and sensory quality parameters of fillets during storage were recorded at 0,15,30,45,60,75,105 and 120 days. The results obtained are presented in this chapter

RESULTS

4.0. Proximate composition of Raw fish :-

1) Moisture - 77.27%
2) Ash - 1.18%
3) Protein - 19.4%
4) Fat - 3.73%

For fillet purpose:-

Avg. fish measurements-

1) Total weight of whole fish - 315 gm, Total length – 23 cm.
2) Total weight of fillet - 120gm = 38 % of total body weight.
3) Total fish waste - 195gm = 62 % of total body weight.

Total weights of fillets per treatment –

1) T0 - 710 gm
2) T1 - 915 gm
3) T2 - 830 gm
4) T3 - 760 gm

Each treatment had 15 fillets and their weights ranged from 30 to 110 gms**.**

4.1 Changes in tilapia fillets during frozen storage period in relation to proximate analysis at first(0) and last (120) days.

4.1.1 MOISTURE

The data on the effect of treatments on moisture (%) content in tilapia fillets during storage period are presented in Table 4.1 and depicted in Fig 4.1.

Table 4.1. Effect of treatments on moisture content of tilapia fillets during frozen storage.

Treatment	MOISTURE(%) content		Mean
	Storage period(DAYS)		
	0	120	
T0	80.00	78.33	**79.16**
T1	79.00	76.00	**77.50**
T2	86.00	82.00	**84.00**
T3	88.33	86.66	**84.54**
Mean	**83.33**	**80.75**	
	S.Em ±	**CD at 5%**	
Treatment (T)	0.301	0.106	
Storage (S)	0.451	0.160	
Interaction (TxS)	0.902	0.319	

It is revealed from the data that moisture slightly decreased at the end of the storage period. The significant differences were observed between the treatments through the storage.

The mean value of treatments for moisture content in all treatments was observed 83.33% on initial (0) day while at the final (120) day of storage period the moisture content was observed to be 80.7%.

The interaction effects between the various treatments and storage were found to be statistically significant. The lowest [79% at initial(0) and 76% at final(120) day] was recorded in the treatment T1, followed by treatment T0 [80% at initial(0),73% at final(120) day]. while highest [88.33% at initial(0) and 80.75% at final(120) day] moisture was recorded in treatment T3, followed by treatment T2 [86% at initial (0),82% at final(120) day].

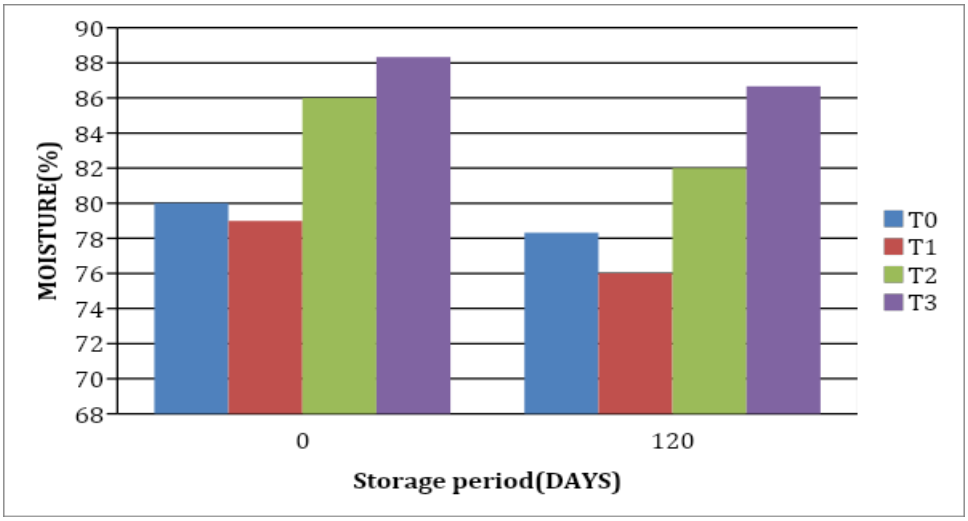

Fig.4.1. Effect of treatments on moisture content of tilapia fillets during frozen storage.

4.1.2 ASH

The data on the effect of treatments on ash (%) content in tilapia fillets during storage period are presented in Table 4.2 and depicted in Fig 4.2.

Table 4.2 . Effect of treatments on ash content of tilapia fillets during frozen storage.

Treatment	ASH (%) content		Mean
	Storage period(DAYS)		
	0	120	
T0	1.22	1.32	**1.27**
T1	1.41	1.53	**1.47**
T2	1.52	1.62	**1.57**
T3	1.13	1.26	**1.28**
Mean	**1.32**	**1.43**	
	S.Em ±	**CD at 5%**	
Treatment (T)	0.013	0.005	
Storage (S)	0.019	0.007	
Interaction (TxS)	0.038	0.014	

It is revealed from the data that ash slightly increased at the end of the storage period. The significant differences were observed between the treatments through the storage

The mean value of treatments for ash content in all treatments was observed 1.32% on initial (0) day while at the final (120) day of storage period the ash content was observed to be 1.43%.

The interaction effects between the various treatments and storage were found to be statistically significant. The lowest [1.13% at initial(0) and 1.26% at final(120) day] was recorded in the treatment T3, followed by treatment T0 [1.22% at initial(0),1.32% at final(120) day] while highest [1.52% at initial(0) and 1.62% at final(120) day] ash was recorded in treatment T2, followed by treatment T1 [1.41% at initial(0),1.53% at final(120) day].

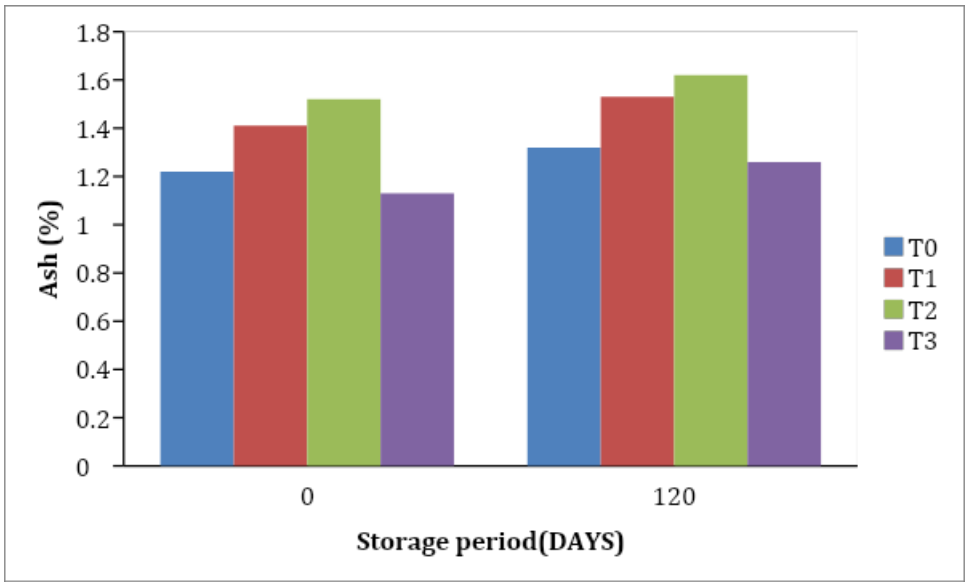

Fig. 4.2 . Effect of treatments on ash content of tilapia fillets during frozen storage.

4.1.3 FAT

The data on the effect of treatments on fat(%) content in tilapia fillets during storage period are presented in Table 4.3 and depicted in Fig 4.3.

Table 4.3. Effect of treatments on fat content of tilapia fillets during frozen storage.

Treatment	FAT(%) content		Mean
	Storage period(DAYS)		
	0	120	
T0	0.65	0.71	0.68
T1	0.71	0.76	0.73
T2	0.76	0.79	0.77
T3	0.64	0.67	0.65
Mean	0.695	0.736	
	S.Em ±	**CD at 5%**	
Treatment (T)	0.002	0.001	
Storage (S)	0.004	0.001	
Interaction (TxS)	0.007	0.002	

It is revealed from the data that fat slightly increased at the end of the storage period. The significant differences were observed between the treatments through the storage.

The mean value of treatments for fat content in all treatments was observed 0.69% on initial (0) day while at the final (120) day of storage period the fat content was observed to be 0.73%.

The interaction effects between the various treatments and storage were found to be statistically significant. The lowest [0.64% at initial(0) and 0.67% at final(120) day] was recorded in the treatment T3, followed by treatment T0 [0.65% at initial(0) ,0.71% at final(120) day] while highest [0.76% at initial(0) and 0.79% at final(120) day] fat was recorded in treatment T2, followed by treatment T1 [0.71% at initial(0), 0.76% at final(120) day].

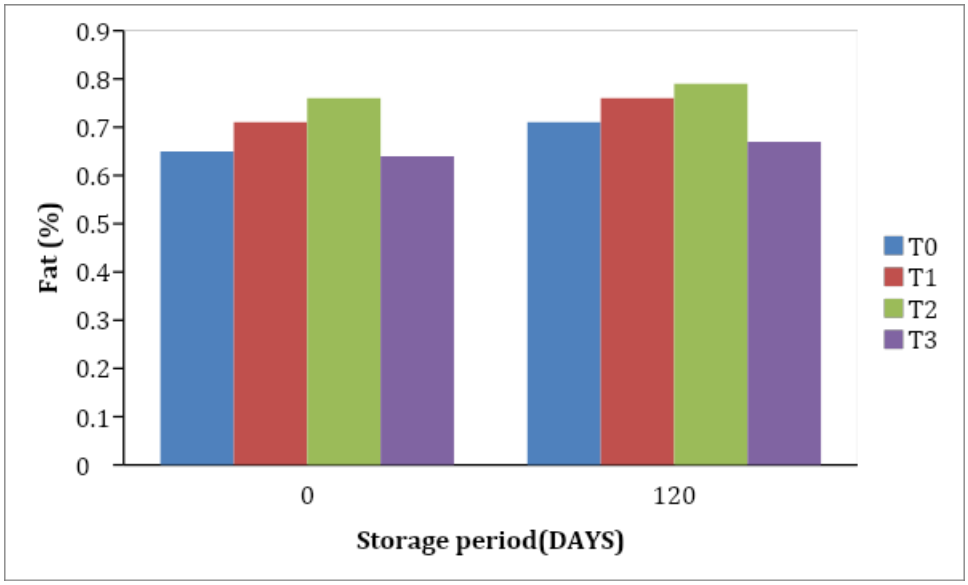

Fig.4.3. Effect of treatments on fat content of tilapia fillets during frozen storage.

4.1.4 PROTEIN

The data on the effect of treatments on protein(%) content in tilapia fillets during storage period are presented in Table 4.4 and depicted in Fig 4.4.

Table. 4.4. Effect of treatments on protein content of tilapia fillets during frozen storage.

Treatment	PROTEIN(%) content		Mean
	Storage period(DAYS)		
	0	120	
T0	17.32	15.33	**16.32**
T1	19.37	16.21	**17.79**
T2	20.50	16.47	**18.48**
T3	18.28	15.23	**16.75**
Mean	**18.87**	**15.81**	
	S.Em ±	**CD at 5%**	
Treatment (T)	0.041	0.015	
Storage (S)	0.062	0.022	
Interaction (TxS)	0.124	0.044	

It is revealed from the data that protein slightly decreased at the end of the storage period. The significant differences were observed between the treatments through the storage.

The mean value of treatments for protein content in all treatments was observed 18.87% on initial (0) day while at the final (120) day of storage period the protein content was observed to be 15.81%.

The interaction effects between the various treatments and storage were found to be statistically significant. The lowest [17.32% at initial(0) and 15.33% at final(120) day] was recorded in the treatment T0, followed by treatment T3 [18.28% at initial(0),15.23 % at final(120) day] while highest [20.50% at initial(0) and 16.47% at final(120) day] protein was recorded in treatment T2, followed by treatment T1 [19.37% at initial(0),16.21% at final(120) day].

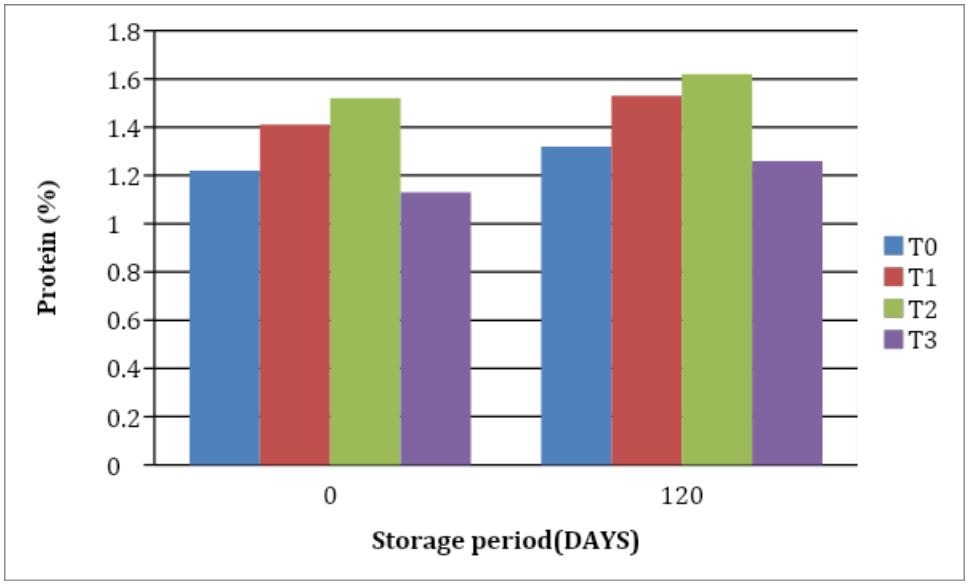

Fig.4.4. Effect of treatments on protein content of tilapia fillets during frozen storage.

4.1.5 NON-PROTEIN NITROGEN (NPN)

The data on the effect of treatments on NPN(%) content in tilapia fillets during storage period are presented in Table 4.5 and depicted in Fig 4.5.

Table 4.5 . Effect of treatments on Non-Protein Nitrogen content of tilapia fillets during frozen storage.

Treatment	NON-PROTEIN NITROGEN(%) content		Mean
	Storage period(DAYS)		
	0	120	
T0	2.22	2.45	**0.51**
T1	2.18	2.29	**0.49**
T2	2.11	2.24	**0.48**
T3	2.33	2.56	**0.54**
Mean	**2.21**	**2.38**	
	S.Em ±	**CD at 5%**	
Treatment (T)	0.003	0.001	
Storage (S)	0.004	0.002	
Interaction (TxS)	0.009	0.003	

It is revealed from the data that NPN slightly increased at the end of the storage period. The significant differences were observed between the treatments through the storage.

The mean value of treatments for NPN content in all treatments was observed 2.21% on initial (0) day while at the final (120) day of storage period the NPN content was observed to be 2.38%.

The interaction effects between the various treatments and storage were found to be statistically significant. The lowest [2.11% at initial(0) and 2.24% at final(120) day] was recorded in the treatment T2, followed by treatment T1 [2.18% at initial(0), 2.29% at final(120) day] while highest [2.33% at initial(0) and 2.56% at final(120) day] NPN was recorded in treatment T3, followed by treatment T1 [2.18% at initial(0), 2.29% at final(120) day].

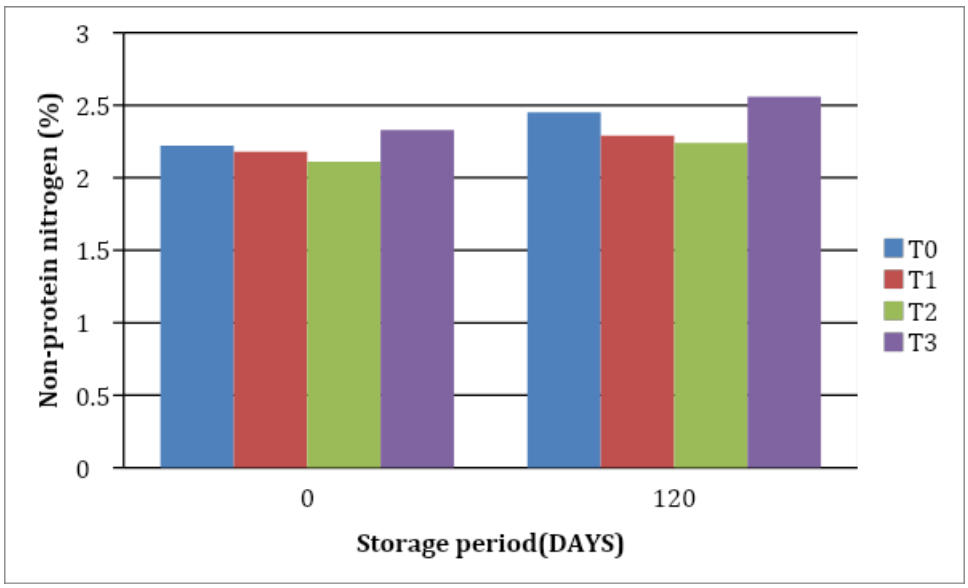

Fig.4.5. Effect of treatments on Non-Protein Nitrogen content of tilapia fillets during frozen storage.

4.2. Changes in tilapia fillets during frozen storage period in relation to physical analysis.

4.2.1 Drip Loss

The data on the effect of treatments on drip loss (%) content in tilapia fillets during storage period are presented in Table 4.6 and depicted in Fig 4.6.

Table 4.6 . Effect of treatments on drip loss content of tilapia fillets during frozen storage.

Treatment	DRIP LOSS(%) content									Mean
	Storage period(DAYS)									
	0	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**	
T0	1.53	1.70	1.56	1.56	1.64	1.70	1.58	1.51	1.80	**1.62**
T1	2.83	2.83	4.45	6.12	6.25	6.12	6.27	6.25	6.03	**5.24**
T2	3.02	4.46	5.27	8.33	8.58	9.15	10.27	9.95	10.16	**7.68**
T3	1.34	1.46	1.56	1.39	1.66	1.37	1.29	1.28	1.32	**1.41**
Mean	**2.18**	**2.61**	**3.21**	**4.35**	**4.53**	**4.58**	**4.85**	**4.75**	**4.82**	
	S.Em ±					**CD at 5%**				
Treatment (T)	0.051					0.018				
Storage (S)	0.076					0.027				
Interaction (TxS)	0.152					0.054				

It is revealed from the data that drip loss slightly increases over the storage period. The significant differences were observed between the treatments through the storage.

The mean value of treatments for drip loss content in all treatments was observed 2.18% on initial (0) day while at the final (120) day of storage period the drip loss content was observed to be 4.82%.

The interaction effects between the various treatments and storage were found to be statistically significant. The lowest (1.34%) was recorded in the treatment T3, followed by treatment T0 (1.53%) while highest (10.16) drip loss was recorded in treatment T2, followed by treatment T1 (6.03%).

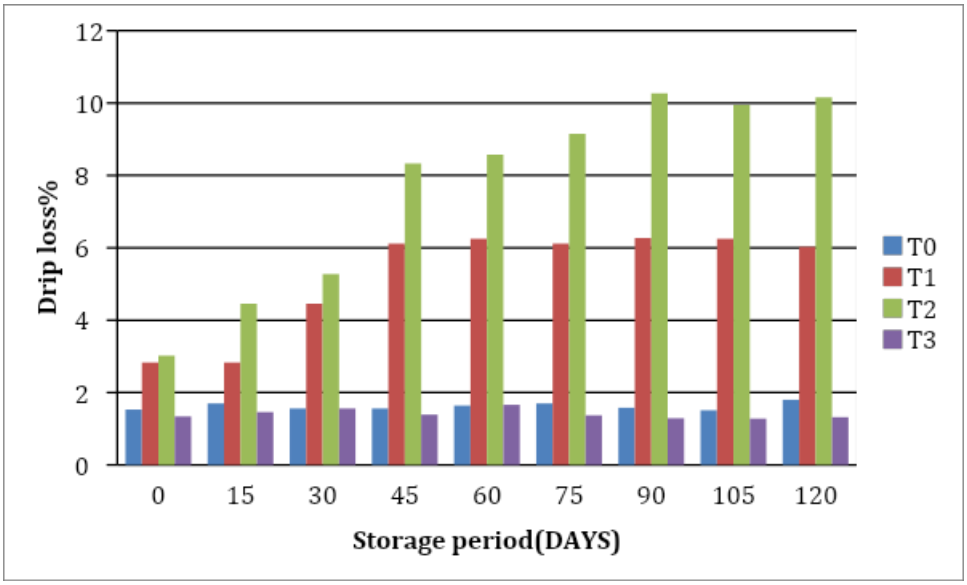

Fig. 4.6 . Effect of treatments on drip loss content of tilapia fillets during frozen storage.

4.2.2 Colour

The data regarding the L* value represents lightness of the fillets by different treatments during storage are presented in Table 4.7 and graphically depicted in Fig.4.

L* value for tilapia fillets colour

Table 4.7 . Effect of treatments on L* value of tilapia fillets during frozen storage.

Treatment	L* value									Mean
	Storage period(DAYS)									
	0	15	30	45	60	75	90	105	120	
T0	80.34	79.53	78.76	77.42	75.76	75.26	74.78	74.45	72.32	76.51
T1	84.12	84.22	83.31	82.44	80.53	79.55	78.85	77.71	75.73	80.72
T2	84.82	84.76	83.64	83.52	82.42	81.16	80.51	79.40	78.18	82.04
T3	78.50	78.31	77.41	76.40	75.49	75.31	74.16	71.41	71.36	75.37
Mean	81.94	81.70	80.78	79.95	78.55	77.82	77.08	75.74	74.39	
	S.Em ±					CD at 5%				
Treatment (T)	0.036					0.102				
Storage (S)	0.054					0.154				
Interaction (TxS)	0.109					0.307				

With respect to storage, an decreasing trend in L* value for colour of fillets was observed in which the mean value of treatments for L* in all treatments was observed (81.94) on initial (0) day while at the final (120) day of storage period the value of treatments for L* was observed to be (74.39).

Interaction between different treatments on storage period was significant at 5% level of significance. The average minimum (71.36) L* value of fillets was recorded in treatment T3 while the maximum (84.82) a* value was observed during storage in treatment T2.

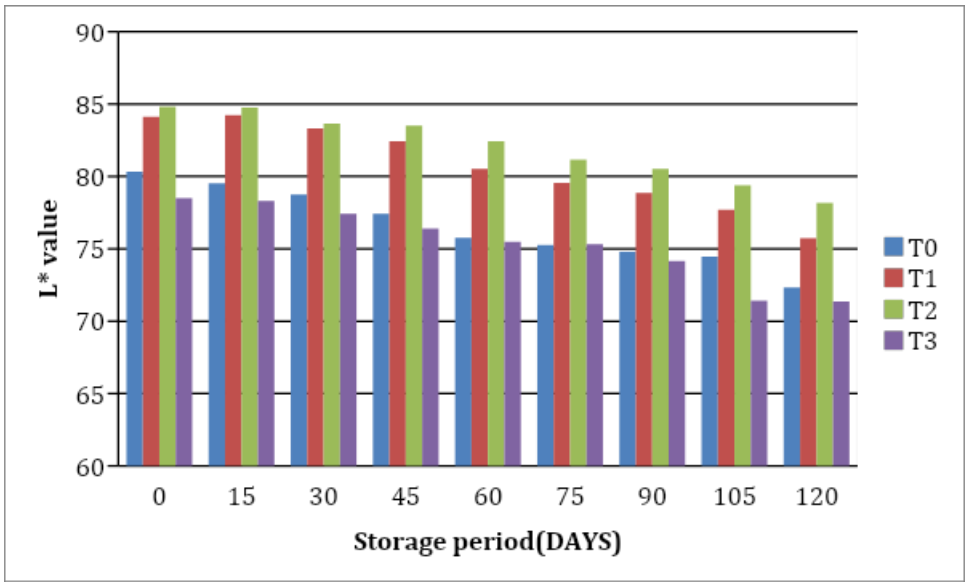

Fig. 4.7 . Effect of treatments on L* value of tilapia fillets during frozen storage.

a * value for tilapia fillets colour

The data on the changes in a* values for fillets due to treatments during storage are presented in Table 4.8 and graphically depicted in Fig 4.8.

Table.4.8 . Effect of treatments on a* value of tilapia fillets during frozen storage.

Treatment	a* value									Mean
	Storage period(DAYS)									
	0	15	30	45	60	75	90	105	120	
T0	6.81	6.76	5.61	4.54	4.53	3.53	3.41	1.52	0.90	4.18
T1	6.72	6.61	5.56	4.51	3.54	3.16	2.55	2.13	1.41	4.02
T2	7.75	7.70	6.65	5.55	4.61	3.71	3.17	2.70	1.60	4.82
T3	5.56	5.36	4.45	4.31	2.31	2.13	1.14	-0.91	-0.81	2.61
Mean	6.71	6.61	5.57	4.73	3.75	3.13	2.57	1.36	0.77	
	S.Em ±					CD at 5%				
Treatment (T)	0.011					0.032				
Storage (S)	0.017					0.048				
Interaction (TxS)	0.034					0.096				

It was observed from the data that the a* value of the fillets showed a decreasing trend during storage.

With respect to storage, an decreasing trend in a* value for colour of fillets was observed in which the mean value of treatments for a* in all treatments was observed (6.71) on initial (0) day while at the final (120) day of storage period the value of treatments for a* was observed to be (0.77).

Interaction between different treatments on storage period was significant at 5% level of significance. The average minimum (-0.81) a* value of fillets was recorded in treatment T3 while the maximum (7.75) a* value was observed during storage in treatment T2.

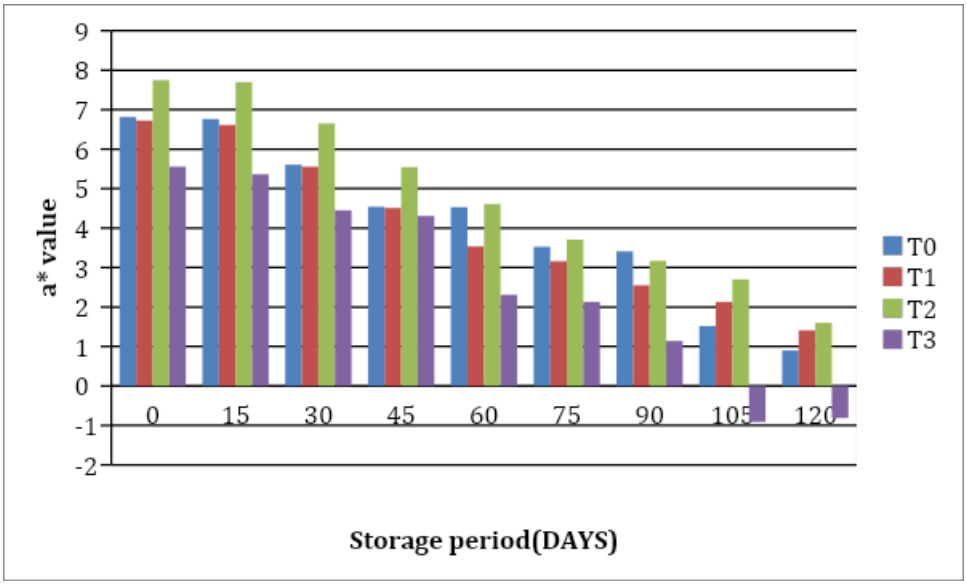

Fig.4.8 . Effect of treatments on a* value of tilapia fillets during frozen storage.

b * value for tilapia fillets colour

The data pertaining to the changes in b* value for fillets are presented in Table 4.9 and graphically illustrated in Fig 4.9.

Table 4.9 .Effect of treatments on b* value of tilapia fillets during frozen storage.

Treatment	b * value									Mean
	Storage period(DAYS)									
	0	15	30	45	60	75	90	105	120	
T0	16.62	16.53	15.43	15.31	14.41	13.41	12.50	10.31	9.31	13.76
T1	17.78	17.18	16.60	14.61	14.17	13.51	12.30	12.13	9.51	14.20
T2	18.81	17.71	17.63	16.60	15.55	14.40	12.80	12.13	10.40	15.11
T3	15.45	15.17	14.80	13.40	13.14	12.13	10.60	10.80	9.37	12.76
Mean	17.16	16.64	16.11	14.98	14.31	13.36	12.05	11.34	9.64	
	S.Em ±					CD at 5%				
Treatment (T)	0.022					0.063				
Storage (S)	0.033					0.094				
Interaction (TxS)	0.067					0.189				

As regard to the storage, there was significant decrease in the b* value for colour in which the mean value of treatments for b* in all treatments was observed (17.16) on initial (0) day while at the final (120) day of storage period the value of treatments for b* was observed to be (9.64).

Interaction between different treatments and storage period on b*was significant at 5% level of significance. The average minimum (15.45) b* value of fillets was recorded in treatment T3 while the maximum (18.81) b* value was observed during storage in treatment T2.

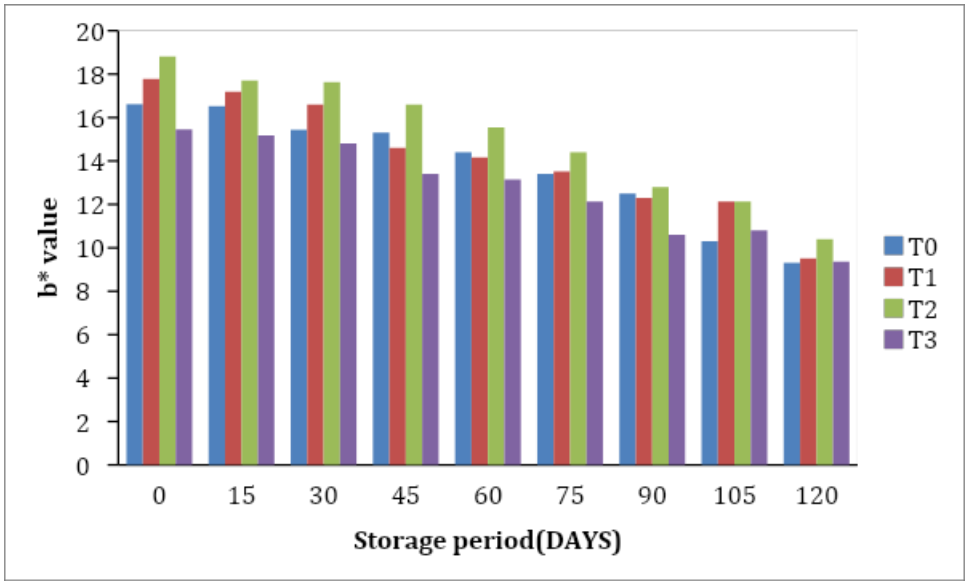

Fig.9 . Effect of treatments on b* value of tilapia fillets during frozen storage.

4.3. Effect of treatments on tilapia fillets during storage period in relation to biochemical analysis.

4.3.1 TVBN (Total volatile basic Nitrogen)

The data on the effect of treatments on TVBN (mg/100g) content in tilapia fillets during storage period are presented in Table 4.10 and depicted in Fig 4.10.

Table 4.10 . Effect of treatments on TVBN content of tilapia fillets during frozen storage.

Treatment	TVBN(mg/100g) content									Mean
	Storage period(DAYS)									
	0	15	30	45	60	75	90	105	120	
T0	12.05	12.32	12.65	15.84	19.90	20.02	20.13	20.15	21.62	17.18
T1	9.88	10.32	11.20	13.32	14.11	15.87	16.07	19.07	19.87	14.41
T2	7.94	8.04	8.88	10.39	12.62	12.97	13.55	14.36	15.89	11.62
T3	12.61	13.21	14.12	16.11	16.36	18.38	20.75	21.61	22.13	17.25
Mean	10.62	10.97	11.71	13.91	15.74	16.81	17.62	18.79	19.88	
	S.Em ±					CD at 5%				
Treatment (T)	0.012					0.004				
Storage (S)	0.018					0.006				
Interaction (TxS)	0.037					0.013				

It could be revealed from the data that TVBN increases over the storage period. The significant differences were observed between the treatments through the storage.

As regards to the storage, there was a significant increase in the TVBN as the storage period increased in which the mean value of treatments for TVBN in all treatments was observed (10.62mg/100g) on initial (0) day while at the final (120) day of storage period the value of treatments for TVBN was observed to be (19.88mg/100g).

The interaction effects between the various treatments and storage were found to be statistically significant. The lowest (7.94mg/100g) was recorded in treatment T2, followed by treatment T1 (9.88mg/100g) while highest (22.13mg/100g) TVBN was recorded in treatment T3, followed by treatment T0 (21.62mg/100g).

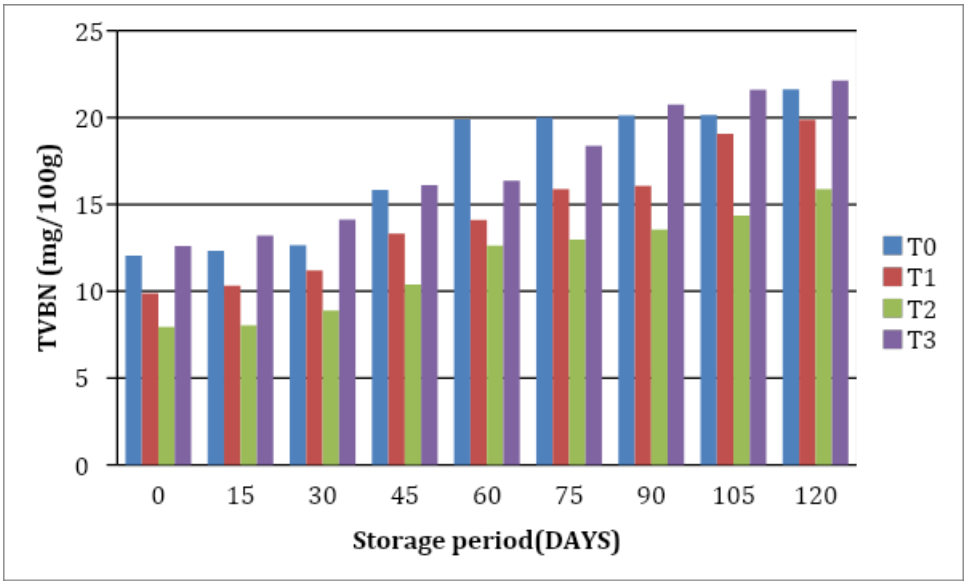

Fig.4.10 .Effect of treatments on TVBN content of tilapia fillets during frozen storage.

4..4. Effect of treatments on tilapia fillets during storage period in relation to Microbiological Analysis.

4.4.1 TPC (Total plate count)

The data on the effect of treatments on TPC content (log_{10}cfu/g) in tilapia fillets during storage period are presented in Table 4.11 and depicted in Fig 4.11.

Table 4.11 . Effect of treatments on TPC content of tilapia fillets during frozen storage.

Treatment	TPC(Log_{10}cfu/gm) content									Mean
	Storage period(DAYS)									
	0	15	30	45	60	75	90	105	120	
T0	5.07	5.18	5.28	5.36	5.39	5.50	5.57	5.64	5.69	**5.41**
T1	4.83	4.97	5.13	5.20	5.24	5.32	5.41	5.44	5.49	**5.22**
T2	4.62	4.69	5.07	5.04	5.15	5.21	5.24	5.31	5.34	**5.07**
T3	5.14	5.24	5.32	5.41	5.44	5.54	5.62	5.69	5.74	**5.46**
Mean	**4.91**	**5.02**	**5.20**	**5.25**	**5.30**	**5.39**	**5.46**	**5.52**	**5.57**	
	S.Em ±					**CD at 5%**				
Treatment (T)	0.008					0.023				
Storage (S)	0.012					0.035				
Interaction (TxS)	0.024					0.070				

It could be revealed from the data that TPC increases over the storage period. The significant differences were observed between the treatments through the storage.

As regards to the storage, there was an increase in the TPC as the storage period was increased in which the mean value of treatments for TPC in all treatments was observed (4.91log_{10}cfu/g) on initial (0) day while at the final (120) day of storage period the value of treatments for TPC was observed to be (19.88log_{10}cfu/g).

The interaction effects between the various treatments and storage were found to be statistically significant. The lowest (4.6log_{10}cfu/g) was recorded in treatment T2, followed by treatment T1 (4.83) while highest (5.74log_{10}cfu/g) TPC was recorded in treatment T3, followed by treatment T0 (5.69log_{10}cfu/g).

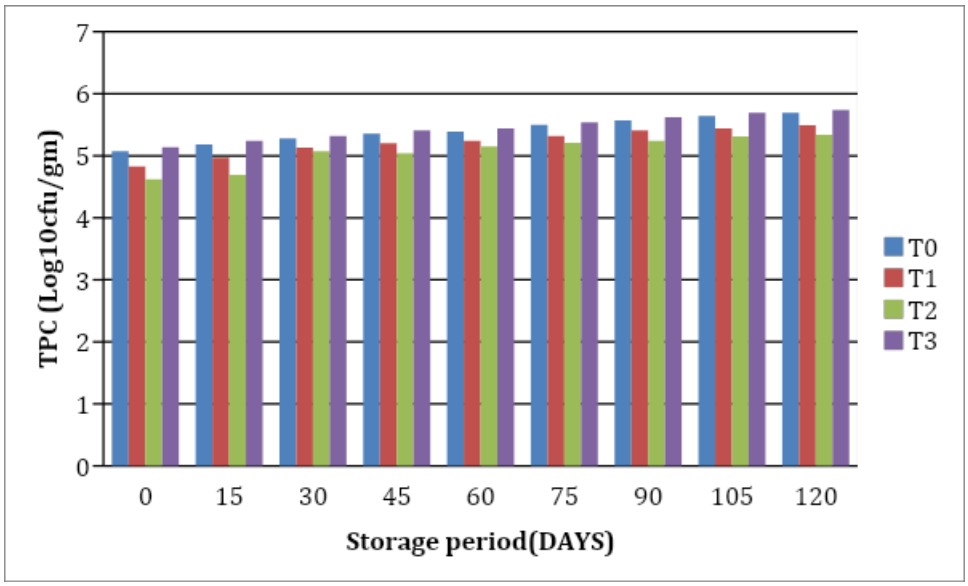

Fig 4.11 . Effect of treatments on TPC content of tilapia fillets during frozen storage.

4.4.2 *E-coli*

Table 4.12 . Effect of treatments on *E-coli* of tilapia fillets during frozen storage.

Treatment	*E-COLI* (cfu/100g)									Mean
	Storage period(DAYS)									
	0	15	30	45	60	75	90	105	120	
T0	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
T1	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
T2	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
T3	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Mean	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	S.Em ±					**CD at 5%**				
Treatment (T)	N.S					N.S				
Storage (S)	N.S					N.S				
Interaction (TxS)	N.S					N.S				

(N.D. :- Not detected, N.S :- Non significant)

In the analysis *E-coli* wasn't detected at any storage period from the initial (0) to final (120) day.

The interaction effects between the various treatments and storage were found to be statistically non-significant.

4.4.3. Effect of treatments on sensory quality parameters of fillets during storage.

The treated fillets were evaluated for their organoleptic characteristics by a panel of experienced judges on a 9 point scorecard on the final(120) day and the results are as below. The data (scores) on sensory evaluation of fillets are presented in Table 4.13 and graphically depicted in Fig 4.13.

Table 4.13 . Effect of treatments on sensory quality parameters of tilapia fillets during Storage.

Treatments	Sensory Score				Overall acceptability
	Colour	Flavour	Texture	Odour	
T0	7.75	7.72	7.74	7.08	7.25
T1	7.52	8.08	8.20	8.00	7.80
T2	8.35	8.60	8.60	8.80	8.60
T3	6.82	7.40	7.23	6.90	7.05
Mean	7.61	7.95	7.94	7.69	7.67
S.Em ±	0.14	0.07	0.15	0.24	0.43
C.D at 5%	0.49	0.25	0.51	0.81	1.34

Colour:-

It was observed that the colour of the fillets under the treatment T2 was liked by judges the most and treatment T2 fetched the numerically maximum (8.35) score, followed by the treatment T1 (7.52), followed by T0. The treatment T3 obtained numerically minimum score for colour i.e. 6.82. The results were statistically significant. Similar observations were reported by (Odoli,2009) in tilapia fillets.

Flavour:-

The treatment T2 was significantly superior to all other treatments and recorded the maximum (8.60) sensory score for flavour. While, the treatment T3 recorded lowest (7.40) sensory score for flavour, followed by the treatment T0 (7.72). The results were statistically significant. The similar observations were reported by (Ozogul et.al.,2009) in anchovy fillets.

Texture:-

The treatment T2 was significantly superior to all other treatments and recorded maximum (8.60) sensory score for texture. While, the treatment T3 recorded the lowest (7.23) sensory score for texture, followed by the treatment T0 (7.74). The results were statistically significant. Identical observation was reported by (Reddy et.al.,1994) in tilapia fillets.

Odour:-

The treatment T2 was significantly superior to all other treatments and recorded maximum (8.80) sensory score for odour. While, the treatment T3 recorded the lowest (6.90) sensory score for odour, followed by the treatment T0 (7.08). The results were statistically significant. Identical observation was reported by (Kautter et.al.,1994) in tilapia fillets.

Overall acceptability:-

The treatment T2 was significantly superior to all other treatments. The treatment T2 received the highest (8.60) sensory score for overall acceptability. The treatment T3 had the lowest (7.05) sensory score for overall acceptability which was followed by treatment T0 (7.25). The results were statistically significant. Identical observation was reported by (Gharbi et.al.,2019) in sardine fillets.

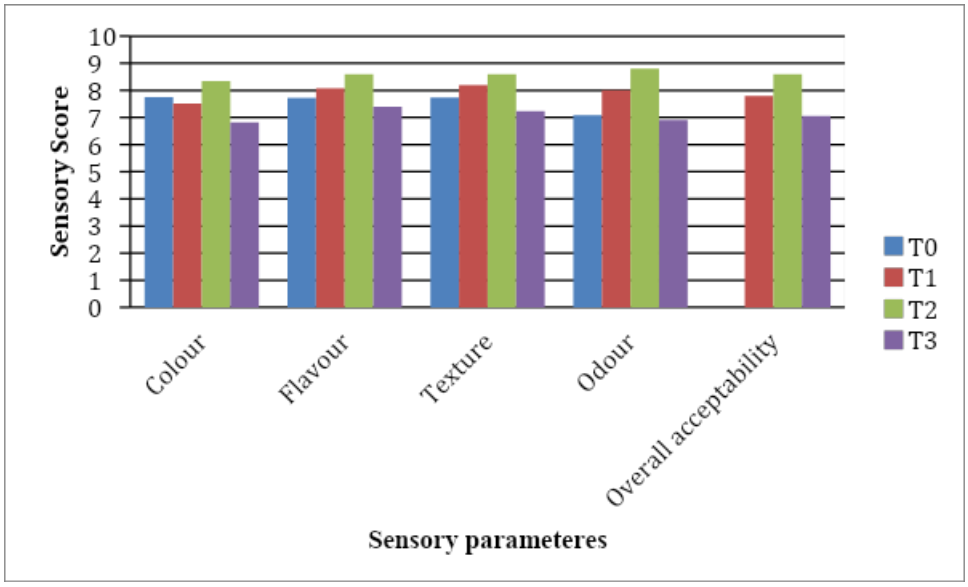

Fig.4.13 . Effect of treatments on sensory quality parameters of tilapia fillets during Storage.

DISCUSSION

The results obtained in the periodical analysis of treated frozen tilapia fillets for 120 days at -18°C related to proximate,physical,biochemical,microbiological parameters are discussed below:-

4.5 Effect of NaCl (salt) and Sodium tripolyphosphate (STPP) treatment on tilapia fillets during frozen storage :-

During the studies on fillets of tilapia treated with NaCl (2%) and STPP (3%) concentration level as pretreatment before freezing for 2hrs in T1,T2,T3. In accordance with present study treatments didn't have any significant effects on storage performance of fillets in relation to drip loss.

The combination of NaCl and STPP at ionic strengths sufficiently high to promote actomyosin dissociation has recently been tested and found to be more effective than brine alone. With South African hake, stored at –12°C, STPP treatment reduced thawdrip to about 3% from 10% to untreated control (14% in water-dipped control); a half-saturated NaCl dip gave 2% thaw-drip but the combination of half saturated NaCl followed by STPP was more effective reducing thaw-drip to about 1%. Half saturated brine was compared with a dip in a mixture of half-saturated NaCl plus half-saturated STPP on cod fillets. Uptake of dip was very variable but greater in the mixed dip. In these samples thaw-drip levels were low initially, about 1.5%, and while levels increased on storage, the mixed dip treatment resulted in considerably less thaw-drip than NaCl alone (Dyer, 1969).

According to Maccallum et al. (1964), cod treated with STPP and twice-frozen were significantly better than untreated twice-frozen samples as indicated by texture and thaw-drip assessments and equal to once-frozen untreated fish. This can be the result of weakening of flesh consistency due to thawing with consequent presentation of increased surface areas of the fillet to the dip solution. There was no clear evidence of enhancement in single and double frozen flounder after the application of STPP dips used commercially in processing this species.

In some species, such as Dover sole *(Microstomus pacifucus)*, Pacific cod (*Gadus macrocephalus)*, halibut (*Hippoglossus stenolepis*) and red snapper (*Sebastodes ruberrimus*), STPP was effective in reducing thaw-drip in comparison with water-dipped controls but was not effective in others, e.g. Chinook salmon (*Oncorhynchus tschawytscha)* (Dyer, 1969).

Antonie et al.,(2000) studied the effects of STPP & NaCl on smoke adsorption and overall quality of cold smoked mullet. Fillets treated with 5 and 10 % STPP or 5% NaCl had 1.20, 1.45 and 2.45 % more moisture, respectively, than control. Treatment with 5 or 10 % STPP both with 5% NaCl absorbed 1.25 and 0.95% more water, respectively; water loss occurred with fillets treated with 15% NaCl, 5 and 10% STPP plus 15% NaCl. STPP enhanced the sensory perception of moistness in treated fish, because of its water retention capability. STPP in combination with NaCl, tended to reduce the relative free moisture level of the product, and at 5 and 10% did not affect the sensory evaluation of saltness.

4.6 The data presented from Table 4.1 to 4.5 and depicted in Fig. 4.1 to 4.5 indicates that the proximate analysis was significantly influenced by different treatments on tilapia fillets.

4.6.1 MOISTURE

As regards to present study on treated tilapia fillets and their frozen storage for 120 days at -18°C, it was observed from the data that in the mean of treatments T3 recorded maximum (84.54%) moisture, followed by the treatment T2 and treatment T0. The treatment T1 recorded minimum (77.50%) moisture which was significantly inferior to the rest of treatments. Thus, it was clear from the data that moisture loss in weight varied with different treatments.

Drip loss during the thawing process might be one of the possible reasons for the decrease in the moisture contents. Furthermore, the loss of moisture content of fish might have been also due to desiccation as well as temperature fluctuation during frozen storage. Fluctuation of cold store temperature (temperature abuse) can be the major cause of dehydration (Emire and Gebremariam,2009). Temperature abuse in the freezer can cause the migration of water vapour from the product to the surface of the container. This defect is sometimes found in commercially frozen foods that have been improperly handled. Similar results were reported for frozen tilapia fish by (Sawant and Magar,1961), (Pawar and Magar,1969) and (Arannilewa et al. 2005). The reduction in moisture content is an advantage since it reduces the fish susceptibility to microbial spoilage, oxidative degradation of polyunsaturated fatty acids, and consequently it improves fish quality and preservation.

Malik et.al (2021) in the frozen storage of five commercial freshwater fish species from river Nile,Sudan showed that moisture content of all studied species was gradually decreased as the storage time progressed. All samples showed the highest moisture contents before freezing (zero time), while the lowest moisture contents were observed at the end of storage period (45 days). Similar trends of reduction were reported by Arannilewa et al. (2005) who stored tilapia fish fillets for up to 60 days at −18°C.

Hafez et.al.,(2019) in frozen storage periods of Nile tilapia found moisture content of raw tilapia samples (A and B) recorded 79.41% and 79.38%, respectively. Concerning the effect of frozen storage conditions, a negligible decrease in moisture content of fish samples (A) at 60 days and little increased at 120 and 180 days. On the other hand, moisture content of fish samples (B) was the same trend except it gradually decreased up to 120 days storage. After 60 days from frozen storage the moisture content decreased to 78.81 and 87.49% for samples from farms A and B, respectively. Moisture content increased to 79.05% in frozen samples from farm A after 120 days. On the other hand the moisture content decreased to 77.01% in farm B frozen samples at the same storage period compared with previous period. At the end of the frozen storage period the values of moisture content were slightly increased in samples from both farms.

Gupta et al.,(2012) found in fish muscle of *Labeo rohita* that showed that initially on day 0,moisture content was found to be 84.74±0.1% and it decreased significantly to the value of 80.84±0.09% on 21st of storage at -12±20°C.The results depicted that there was 1.08%, 2.70% and 4.60% decrease in moisture content on 7th, 14th and 21st day respectively.

Alasalvar et al (2002), Orban et al (2002) and Bekelvik et al (2005) reported a decrease in total moisture content in sea bass (*Dicentrarchus labrax)* fillets during frozen storage. This decrease in moisture content was attributed to the sublimation of ice in frozen storage and drip loss during the thawing process by them.

Kandeepan & Biswas (2007) reported a decrease of 1.57% in total moisture content in buffalo meat after seven days of frozen storage. In contrast (Zamir et al (1998) in crab; Bao et al (2007) in arctic Charr (*Salvelinus alpinus*) and Siddique et al (2011) in *Puntius* sps found an increasing trend in moisture content. Zamir et al (1998) attributed this increase to the loss of water holding capacity of tissue.

4.6.2 ASH

With respect to the present study on treated tilapia fillets and their frozen storage for 120 days at -18°C, it was observed from the data that the mean of treatment T2 recorded maximum (1.57%) ash, followed by the treatment T1 and treatment T3. The treatment T0 recorded a minimum (1.27%) ash which was significantly inferior to the rest of treatments.

Similar results were observed by (Inass malik et.al.,2021) in five commercial fishes frozen at -18˚C for 45 days.Loss of water in any food substances produce an uneven increase in the percentage of other nutrients,therefore, the consequential increase in ash content during freezing might be due to the decrease in moisture and protein contents of the studied fish samples.(Castrillon et al., 2008)

Emire et al.,(2013) found that during 90 days of storage for ash at -18°C. The initial moisture and ash contents decreased significantly ($P < 0.05$) from 79.87 and 0.98% to 78.50 and 0.89%, respectively, at the end of the storage period. A decrease in ash content was also reported by (Beklevik et al.,2005) for sea bass fillets during 60 days of storage under frozen condition.

Sharma et al.,(2012) found in fish muscle of_*Labeo rohita*_that ash content decreased significantly from 1.79±0.01% on day 0 to 1.36±0.03% on 21st day of storage at -12±20C .There was a total decrease of 5.58%,12.29% and 24.02% on 7th,14th and 21st day of storage respectively.

Beklevik et al,(2005) while working on sea bass fillets; Okoyo et al,(2009) on Nile perch and Emire et al.,(2009) on Tilapia (*Oreochromis niloticus*) have reported a decrease in total ash content during its frozen storage.The decrease in ash and moisture content was attributed to the drip loss during the thawing process.But Aannilewa et al.,(2005) observed that the ash content remained almost the same throughout the 60 days of frozen storage of tilapia.

4.6.3 FAT

In accordance with the present study on treated tilapia fillets and their frozen storage for 120 days at -18°C, it was observed from the data with respect to mean of the treatments T2 recorded maximum (0.77%) fat, followed by the treatment T1 and treatment T0. The treatment T3 recorded a minimum (0.65%) fat which was significantly inferior to the rest of treatments. The tilapia fish used in this work is considered lean fish (< 2% fat). The results agreed with (Khidhir.et al.,2015) who reported that the values of fat content of Rainbow trout and tilapia muscles ranged between 1.05 - 1.29%, they also showed that the fat content means values were 1.665; 1.048; 2.008 and 1.523% for Myanmar, Flander, Hasoon and White fish fillet respectively.

Similar increases in fat content during storage have been reported in tilapia fish fillets by (Emire & Gebremariam, 2010) and sea bass fillets (Özyurt et al., 2005). The resultant increased fat content can be due to the fact that there is an inverse relationship ($r = -0.99$) between the moisture and lipid contents of fish flesh (Inhamuns & Franco, 2001;Oliveira et al., 2003;Begum et al., 2012).

The reason for the fat increase is lipid oxidation which a major cause of deterioration for many foods containing fats and oils. The large amount of polyunsaturated fatty acid moieties found in fish lipids makes them highly susceptible to oxidation by an autocatalytic mechanism (Smith and Hui 2004). Lipid deterioration limits shelf life of fish. Consequently, lipid oxidation causes loss of flavour and nutrition, and creates toughening and other texture problems (Aubourg & Medina,1999). The result is an unpleasant odour and flavour called rancidity. The oxidation of lipids plays a very important role in the spoilage of both lean and fatty fishes.

Emire et al.,(2013) found that in frozen tilapia, the fat content of tilapia fish fillets stored for about 3 months under frozen condition increased significantly ($P < 0.05$) from 0.37% to 0.56%.This was due to the fact that there was an inverse relationship between the moisture and lipid contents of fish flesh.

According to Beklevik et al.,(2005) the lipid ratio of sea bass fillets was 1.22% at the beginning of the storage and it was reported as 2.28, 2.86, and 3.58% in the 3rd, 6th, and 9th month of storage, respectively. Except for the slight differences, which may be due to the differences in type of fish species, fish size, lipid content, feeding state at the moment of capture,

the catching season and geographical location, the observed changes in fat content occurring during tilapia fish fillets stored under frozen condition were consistent.

Tokur (2000) also reported an increase in lipid content occurred during frozen (-18˚C) storage of rainbow trout. The fat content of tilapia fish fillets stored under frozen condition decreased from day 15 to day 30 as well as from day 45 to day 60, this being followed by an increasing trend up to the end of the storage period.

Sharma et al.,(2012) found in fish muscle of *Labeo rohita* that the fat content decreased significantly ($p \leq 0.05$) from day 0 i.e. 3.86±0.03% to 3.00±0.03% on 21st day. There was a 5.44%, 15.80% and 22.27% decrease in fat content on 7th, 14th and 21st day respectively during frozen storage of 21 days at -12±20˚C.

Arannilewa et al,(2005) in Tilapia and Siddique et al,(2011) in *Puntius* sps found a significant loss in total fat content when stored at low temperature.

4.6.4 PROTEIN

In the case of present study on treated tilapia fillets and their frozen storage for 120 days at -18°C, it was observed from the data that the with respect to mean of treatments T2 recorded maximum (18.48%) protein, followed by the treatment T1 and treatment T3. The treatment T0 recorded a minimum (16.32%) protein which was significantly inferior to the rest of treatments.Thus, it was clear from the data that protein varied with different treatments.

Similar results were observed by Arannilewa.et al,(2005) who reported that protein content of fresh Nile tilapia (*Sarotherodon galiaenus*) was 60.65%(dry wt.) and decreased to 57.7 and 43.7% after 1 and 2 months of frozen storage, respectively. After 120 days of frozen storage, protein content decreased to 15.81% from 18.87%. Also Siddique.et al.,(2011) reported a significant decrease in protein content during frozen storage of fish. The reasons for decrease can be as follows -

The relatively high crude protein in freshwater fishes could be attributed to the fact that fishes are a good source of pure protein. Indeed the protein content of the species is higher than that of the egg yolk (15%) reported by CFCD(China food composition database",2002).Normal range values of protein in fish (15-25%), and the variation in protein content could be due to the variation in species, feed availability, sexual maturity, spawning, season of catching and processing methods. Jenkelunas (2013) showed that muscle protein stored at freezing temperatures will incur damage due to ice crystal formation. As water molecules freeze, they begin to migrate away from the protein into ice crystals, thus exposing hydrophobic and hydrophilic areas and allowing intra- and intermolecular cross linkages. Also he reported that the native structure of a protein is usually the most stable conformation under normal physiological conditions. However, during frozen storage proteins are susceptible to denaturation. The rate at

which muscle protein denatures varies among species. Fish myofibrillar proteins, despite having similar amino acid composition to the proteins from mammalian fish species, are much less stable than the latter.During the early stages of autoxidation, free radicals and relatively stable hydro peroxides are formed, which subsequently react with proteins, causing polymerization of proteins and destruction of amino acids (Emire and Gebremariam, 2009). Fish proteins can also be impaired by rancidity. During the early stages of autoxidation, free radicals and relatively stable hydroperoxides are formed, which subsequently react with proteins, causing polymerization of proteins and destruction of amino acids (Danopoulos and Ninni 1972).

According to (Xiong 1997, Zamir et al,1998 and Saeed & Howell,2002) proteins exposed to oxidising environments are very susceptible to chemical modification, such as amino acid destruction, peptide scission and formation of protein-lipid complexes that results in decrease in protein content. Several researchers observed that protein decreased during frozen storage and this reduction in protein was ascribed to denaturation (Saliu,2008;Arekemase et al., 2010; Ekpenyong & Bok,2012) and to changes in the proportion of chemical composition and protein breakdown (Kjærsgård et al.,2006). Also, there may be leaching of nitrogen during thawing which in turn causes a reduction in the protein content (Arannilewa et al.,2005; Akter et al.,2012;). Furthermore, fish undergoes rapid protein degradation due to the action of endogenous and bacterial enzymes after death of fishes (Saeed & Howell, 2002). Deterioration of fish quality due to protein denaturation can therefore be checked by using as low temperature as possible, preferably at −18°C. Storage temperature and time have a great impact on the degree of protein denaturation (Herrero et al.,2005). The shelf life of fish products, therefore, is markedly extended when products are stored at low temperatures.Other possible reasons for decrease in protein content might be the loss of ammonia (NH3), volatile amines, conversion of nitrogen to other nonprotein nitrogen molecules, amino acid destruction, and formation of protein–fat complexes that results in decrease in protein content (Saeed & Howell, 2002).

Sajjan et al.,(2015) found in deboned tilapia that protein content of the fish meat sample changed significantly from 14.30 to 13.20 in frozen period.Following death, fish undergo rapid protein degradation as a result of endogenous bacterial enzymes.Fish proteins can also be impaired by rancidity. Also Emire et al.,(2013) found that in frozen tilapia the highest protein content (18.52g/100g) was recorded for fresh fish sample and the least protein content (17.25g/100 g) was recorded for fish sample stored for 90 days.A number of factors, including slow freezing and variability of storage conditions, caused this denaturation. Rate at which denaturation occurs depends largely on freezing temperature. The protein content of the fish sample changed significantly ($P < 0.05$) from 18.52±0.08% to 17.25±0.09%.

Sharma et al.,(2012) found in fish muscle of (*Labeo rohita)* that highest protein content (15.93±0.04%) was recorded for fresh (unfrozen) fish samples and the least protein content (13.06±0.04%.) was recorded for fish samples stored for 21 days at -12±20°C. A significant

percent decrease ($p \leq 0.05$) was found in total protein content i.e. 5.77%, 11.23% and 18.01% on 7th ,14th and 21st day of storage respectively.

Bekelvi et al.,(2005) in sea bass (*Dicentrarchus labrex*) and Siddique et al.,(2011) in *Puntius* sps reported significant decrease in protein content during frozen storage. They attributed this protein loss due to the leaching effect of amino acids and water soluble protein leaching out with melting ice.

4.6.5 NON-PROTEIN NITROGEN -

According to present study on treated tilapia fillets and their frozen storage for 120 days at -18°C, it was observed from the mean of data of the treatments that T3 recorded maximum (0.54%) Non-Protein Nitrogen,followed by the treatment T0 and treatment T1. The treatment T2 recorded a minimum (0.48%) Non-Protein Nitrogen which was significantly inferior to the rest of treatments. Thus, it was clear from the data that non-protein nitrogen varied with different treatments.

The results comply with Joseph et al.,(1988) who reported in frozen cuttlefish fillets that the NPN was around 24 % of total nitrogen for the first 10 months storage and the percentage moisture was 76-77 % during the same period. Afterwards a decrease in both the values was noticed. This indicated loss of water holding capacity of the muscle which resulted in increased drip loss and lower moisture content. The amount of NPN fraction leached in the drip also increased concurrently.The changes may be due to leaching of soluble compounds in the muscle.

Abdel,(2001) reported in frozen Nile karmount that NPN for the samples increased during frozen storage.The mean time at zero day was (271.4mg/100g) and after 6 months of frozen storage it increased to (403.2mg/100g).

Finne et al.,(1980) reported NPN for frozen sheepshead (0.29%),black drum (0.30%),sand trout (0.36%),mullet (0.25%), tilapia (0.25%).

Similar results were observed by Salama (2006) in fresh Nile tilapia stored at -20˚C for 6 months in polythene bags,ice glaze film

4.7. DRIP LOSS

4.7.1 The data presented in Table 4.6 & depicted in Fig 4.6 indicates that the physical analysis was significantly influenced by different treatments on tilapia fillets.

With respect to the present study on treated tilapia fillets and their frozen storage for 120 days at -18°C, it was observed from the data that the treatment T2 recorded maximum (7.68%) drip loss, followed by the treatment T1 and treatment T0. The treatment T3 recorded a minimum (1.41%) drip loss which was significantly inferior to the rest of treatments.Thus, it was clear from the data that drip loss varied with different treatments. Similar results were observed by Erkoyuncu et.al.,(2004) in rainbow trout where usage of sodium polyphosphate and sodium

metaphosphate with NaCl was not effective in preventing drip loss.This condition may be due to insufficient penetration of the chemical used into tissue of fish a white and dry layer was seen on the surface of trout during the frozen storage may be phosphate solution that was not absorbed and than deposited.Also Dyer et al.,(1964) have found that treatment with sodium tripolyphosphate (STPP) had no effect on drip loss although the net weight of the muscle increased, while MacCallum et al.,(1964) found that it was only effective in some instances.

The ability of the fish muscle to retain its natural water, and therefore its juiciness, is one of the quality criteria of fresh fish, especially from a consumer perspective.Drip loss has been linked to partial denaturation of proteins during processing, which causes lower WHC. The drip percentage is directly related to the increased refrigerator storage time; this increase has been higher in slow-freezing samples compared to their quick-freezing counterparts ($p<0.05$). Several studies have suggested that drip increase and the fish weight loss occur after defrosting. With raising the freezing speed and time, the drip percentage diminished. It is because of the difference in the location of the ice crystals, their size and shape and consequently the physical damages to the muscle fibers. Drip loss is defined as the nutrients from and value of fish, which is potentially available for human consumption, but fails to be consumed or sold as products. Drip during transportation of product from source to final consumer or storage may result in weight below legally allowed tolerance even if it is wholesome and fit for consumption.(FAO ,1996).

Lauzon et al.,(2012) in air packed (AP) and modified atmospheric packed (MAP) frozen tilapia fillets reported that higher drip was observed in MA-packaged groups with T3 recording higher values that were significantly different from T2 ($P < 0.05$) on day 13, 16, and 20 for T2 and T1.With respect to packaging atmosphere, higher drip was recorded with storage at refrigeration (1°C) than superchilling (1°C). Also Sivertsvik et al.,(2003) reported that superchilled storage leads to less exudation probably due to high water-holding capacity at low temperature. It is therefore evident that air-packaged tilapia fillets stored at 1°C (T2) showed better quality related to analysed physical properties.

Karami et al.,(2018) reported in frozen red tilapia that the growth of the drip for slow-freezing samples was higher than that of quick freezing ($p<0.05$). In slow freezing of Red Tilapia samples, the drip percentage during 6 months storage increased from 4.8-11.4%, while it rose from 2.1-6.1% in the quick-freezing samples. As it can be observed, the increase in the drip volume was directly related to the refrigerator storage time ($p<0.05$) & in the study of Alizadeh et al.,(2007) quick freezing on salmon, the drip percentage increased to 7.75% in the quick-freezing method ($p<0.05$).

4.7.2 L*, a* and b* colour value :-

The data regarding L* value of fillets is represented in table 4.7 and figure 4.7. L* determines lightness of fillets. As regards to our research on treated tilapia fillets and their frozen storage for 120 days at -18°C, there is a significant decrease of L*values. It was observed from data that on an average maximum (82.04) L* value was observed in treatment T2 which is followed by treatment T1 (80.72) .The minimum (75.37) L* value was observed in the treatment T3 followed by the treatment T0. The present data shows that the lightness of fillets significantly decreased in treatments in the storage period.

The data regarding a* value of fillets is represented in table 4.8 and figure 4.8. As regards to present study on treated tilapia fillets and their frozen storage for 120 days at -18°C, there is a significant decrease of a*values. It was noticed from the results that a* value was significantly varied with the treatments. The maximum (4.18) a* value for fillets was observed in the treatment T2 which was significantly at par with treatment T0. The average minimum (2.61) a* value recorded in treatment T3 .

The data regarding b* value of fillets is represented in table 4.9 and figure 4.9. As regards to present study on treated tilapia fillets and their frozen storage for 120 days at -18°C, there is a significant decrease of a*values.There was a significant effect of treatment on b* value for fillets colour. It was observed from the data that significantly maximum (15.11) b* value was observed in the treatment T2 which was at par with treatment T1. The minimum (12.76) was recorded in treatment T3.

Similar results were observed by Sajjan et.al.,(2015) in deboned tilapia fish in which the highest colour values (L *= 52.10, a *=2.08 and b *= 12.30) were observed in the fresh deboned samples that were not subjected to frozen storage, while the least colour value (L *= 45.93, a *= 0.28 and b *= 10.86) was recorded for a deboned meat sample that was stored for 90 days. With the increase in the storage period the colour values were decreasing. Also the similar trend was observed by Kermit and Jerry (1991) on characterization and frozen storage stability of cod mince subjected to mechanical separation of seal worms or cod worm.

The difference in colour values in samples probably could be attributed to dissimilarities in properties of the light-scattering cellular matrix, as mentioned by (Little et al.,1979). Types of samples and nature of sample presentation to the instruments during reflectance measurement have been reported to strongly influence colour parameters for the flesh of salmonids (Little and Mackinney, 1969; Schmidt and Cuthbert, 1969; Choubert, 1982). Frozen storage resulted in extensive colour changes of fillets, causing them to become lighter, more red and yellow, and shifting the hue more to red, compared with the initial samples. Change in colour to a more reddish hue after frozen storage and thawing of fillets may have been due to changes in light absorption and scattering caused by freeze denaturation of the protein of the trout fillets, according to the Kubelka-Munk Colorant Layer Concept (Francis and Clydesdale, 1975).

Alternatively, the changes may have been caused by exposure of carotenoids to a more polar, water-rich environment by release of intracellular liquids. Increasing amounts of water added to astaxanthin dissolved in acetone resulted in a shift in hue toward red. Storage temperature and oxygen concentration are important in determining rate of loss of carotenoid pigments during processing and storage of seafood products (Lusk et al.,1964).

Kong et al.,(1991) in frozen rainbow trout fillets reported that carotenoid concentration of salmonid flesh was reflected in instrumental redness (a*) of fish tissue. A significant relationship between yellowness (b*) and carotenoid concentration was in contrast.Carotenoids deposited in trout flesh, regardless of sources and the environmental factors under which the fish were reared, were considerably stable for up to 6 months under storage conditions of -20 or -80°C and vacuum-packaging. On the other hand,Chen et al.,(1984) found that carotenoids deposited in the flesh of rainbow trout were stable (90% retention) in air-packed samples at l2°C, while oxygen-evacuated and CO-enriched packaging caused adverse colour stability.

Rapid fading of canthaxanthin in rainbow trout flesh during storage at - 12°C and - 30°C was reported by (Pozo et al.1988).Also Schmidt and Cuthbert (1969) found no appreciable change in colour after 105 days frozen storage (- 30°C) of sockeye salmon (*Oncorhynchus nerka*).

4.8. TVBN (Total volatile basic Nitrogen)

4.8.1 The data presented in Table 4.10 and depicted in Fig 4.10 indicates that the biochemical analysis was significantly influenced by different treatments on tilapia fillets.

The data observed in present study on treated tilapia fillets and their frozen storage for 120 days at -18°C there is a significant increase of TVBN values. It revealed that the treatment T3 recorded a maximum (17.25mg/100g) TVBN,followed by the treatment T0. The treatment T2 recorded a minimum (11.62mg/100g) TVBN which was significantly inferior to the rest of treatments.Thus, it was clear from the data that TVBN varied with different treatments..Similar results were observed by Nazemroaya et al.,(2011) in samples of Spanish mackerel (*Scomber commersoni*), who obtained a total increment of 15 mg TVB-N/100g after 6 months of storage at -18 ºC. While, in samples of a lean fish as the sea bass (*Dicentrarchus labrax)* the TVBN total increment, after nine months of frozen storage at -18 ºC, was 2.87 mg /100 g, obtained by (Özyurt, Polat and Tokur,2007). The reason for the increase may be due to the degradation of nitrogen-containing compounds, such as proteins, to various amines.

A level of 35 mg/100 g has been considered the upper limit, above which fishery products are considered spoiled (Connell, 1990). Nevertheless previous studies have shown how TVBN quantities do not always indicate the beginning of the spoilage process, even when those values exceed the tolerance limit (Shakila et al., 2003). (Köse and Erdem,2004) found that the TVBN values do not follow a sensorial evaluation. Besides it is suggested that the TVBN value

depends on the species, catching season and region, age and sex of fish (Kilinc and Cakali, 2005) while the TVBN value limit remains constant for all the teleost fishes.

Since the TVBN increase during frozen storage is principally related to the breakdown of the trimethylamine oxide (TMAO) from enzymatic activity, the initial amount in flesh of this component is very important. The TMAO content varies with the season, size and age of fish, as well as environmental conditions to which the animal is subjected (Huss, 1995). Castell, Smith and Neal (2011) also detected that fish species stored without the dark lateral muscle presented a greatly reduced amount of the products developed by the TMAO breakdown. TVB-N includes TMA, DMA, ammonia and other volatile basic nitrogenous compounds related to seafood spoilage.Ozogul et al,.(2010) reported that the total volatile basic nitrogen can be used only as an indicator of fitness for consumption rather than as an index of freshness throughout the storage life of fish.

Kuley et al.,(2009) in frozen marinated anchovy fillets reported that at the beginning of storage, the TVB-N value was 11.90±0.70 mg/100g of flesh. During the storage, TVB-N value significantly ($P<0.05$) increased and reached 16.91± 0.66 mg/100g of flesh at the end of storage (7 months). Similarly, TVB-N in anchovies marinated with 4% acetic acid and stored at 4 °C increased from 9.8 to 14 mg/100 g during the storage of 8 months.

Yang et al.,(2016) in air packed and modified atmospheric packaged frozen tilapia fillets reported that initial TVB-N value is 7.81×10 mg/100g of muscle, and is significantly increased to 22.05 mg/100 g and 21.74 mg/100g at 11th and 14th day of AP and MAP, respectively, during freezing-point storage. For many fish species, the TVB-N contents increase curvilinear or linearly with time. The shelf lives of the AP and MAP fillets are less than 11th and 14th day, respectively, according to the upper limit TVB-N value of 20 mg/100 g muscle. This difference is probably caused by the CO_2 in the MAP, which inhibits the growth of microorganisms, thus effectively slowing both protein degradation and the increase of TVB-N.TVB-N were unacceptable in AP at 11 days, but remained acceptable in MAP until 14 days.

Kaba and Corapci,(2014) reported that with the increase of CO_2 ratio, the TVB-N value of the product decreased, suggesting that the CO_2 was responsible for delaying the formation of TVB-N by restricting bacterial growth. Liu et al.,(2010) suggested that TVB-N was correlated well with storage time ($r=0.98$), sensory acceptability ($r=-0.93$) and bacterial counts ($r>0.90$) during tray-packed storage at 0°C.

In air packed and modified atmospheric packed frozen tilapia fillets depicted slow TVB-N accumulation in all sample groups (<25 mg N/100 g) during storage at 1°C and 1°C .Nonetheless at the end of storage time (day 20), T1 was different (21 mg N/100 g) to other groups but within concentration of 30 mg/100 g, above which fish is considered unfit for

consumption. This is because changes in some of volatile nitrogen bases are influenced most importantly by microorganisms which were >log 8 CFU g^1 in T1 on day 20.(Arason et al.,2012)

Mano et al.,(2013) in refrigerated fillets of tilapia (*Oreochromis niloticus*) packed in modified atmosphere and gamma-irradiated reported that it consistently indicated an increasing deterioration of the fillets with storage time in all treatments, demonstrating a progressive reduction in the quality of the samples during the storage period. However, the samples treated with irradiation, MAP, or a combination of both treatments (T2,T3,T4,T6,T7,T8) deteriorated more slowly and had longer shelf lives in comparison with the control samples, demonstrating the positive effects of MAP, gamma radiation, and the two conservation methods combined.

4.9. TPC (Total plate count)

4.9.1 The data presented in Table 4.11 and depicted in Fig 4.11 indicates that the microbiological analysis was significantly influenced by different treatments on tilapia fillets.

Regarding to present study on treated tilapia fillets and their frozen storage for 120 days at -18°C, there is a significant increase of TPC values It was observed from the data that the treatment T3 recorded a maximum (5.46 $\log_{10}$cfu/g) mean TPC, followed by the treatment T0. The treatment T2 recorded a minimum (5.07$\log_{10}$cfu/g) mean TPC which was significantly inferior to the rest of treatments.Thus, it was clear from the data that TPC varied with different treatments. Similar results were observed by Gupta et.al.,(2012) reported in *(Labeo rohita) i*n which TPC increased from initial load of 2.04±0.2 log cfu/g to 5.10±0.2 log cfu/g after 21 days of frozen storage. The results clearly revealed that the microbial growth was more with increasing storage period. Similar increment on total bacterial load in at low temperature storage was reported by (Lawire,1998;Obemeata et al,2011).The reason for microbial growth promoting effect of moisture on microbes in meat stored in chiller to the is due to less acid enzymatic reactions of fish flesh.

Nidoni et al.,(2015) reported that the total plate count of deboned tilapia fish meat stored for 90 days at 18±2°C under frozen condition,the initial total plate count load was found to be 2.51×10^6 cfu/g and at the 90 days of storage, it was observed to be 0.98×10^6 cfu/g. The decrease in total plate count of tilapia fish meat decreased by two logs (from 10^6 to 10^4 cfu/g) up to 60 days whereas, 90 days onwards there was an increase in total plate content.However, after 90 days of storage, the microbial load started to increase, which indicated the quality deterioration after 90 days of storage during frozen condition.

Duan et al.,(2009) reported in frozen lingcod fillets that microorganisms grew fast in cold stored fish fillets. The initial total plate count of fresh fish fillets was 2.18 log CFU/g, but the population increased to 6.21 log CFU/g by the end of the 1st week, and reached 7.55 log CFU/g by the end of the 2nd week storage in uncoated samples. Chitosan coatings significantly lowered the total plate count in fish samples ($P < 0.05$), with 0.60–1.19 log CFU/g reductions being

obtained in the coated samples, and the total plate counts of coated samples were below 10^7 CFU/g during the first 2 weeks of cold storage. Jeon et al (2002) reported that chitosan coatings resulted in 2–3 log reductions in total plate counts of herring and cod samples after 12 days of refrigerated storage.

4.9.2. *E-coli*

The data regarding *E-coli* of tilapia fillets changes during frozen storage is represented in table 4.12.

As regards to our research on treated tilapia fillets and their frozen storage for 120 days at -18°C, *E-coli* was absent in all analysed samples in the frozen storage period. It determines that E-coli is sensitive to freezing and frozen storage.

Also Nidoni et al.,(2015) reported in mechanically deboned tilapia fish (*Oreochromis mossambicus)* meat under frozen condition in which the present study showed that the *E-coli* were not detected throughout the storage period.Therefore,the frozen stored tilapia fish meat samples retained their quality acceptable for human consumption till 90 days of storage.

CHAPTER V : SUMMARY AND CONCLUSIONS

The present study entitled, “Freezing and Storage performance of Tilapia Fillets” was undertaken in the Department of Post-Harvest Management of Meat,Poultry,Fish during the year 2021-2022. The experiment consisted of four different treatments on fillets.The experimental data was analysed statistically using Factorial Completely Randomised Design (FCRD). The observations of proximate, physical,bio-chemical,microbiological and sensory quality parameters of fillets during storage were recorded at 0,15,30,45,60,75,90,105 & 120 days of frozen storage.

5.1 Summary

5.1. Effect of treatments on tilapia fillets during storage period in relation to proximate analysis at first(0) and last (120) days.

5.1.1 Moisture (%)

Moisture content in tilapia fillets decreased slightly at the end of storage period of 120 days. Among different treatments,treatment T3 recorded maximum (84.54%) moisture, followed by treatment T1 which recorded minimum (77.50%) moisture which was significantly inferior to the rest of treatments.The interaction effects between various treatments and the storage were statistically significant.

5.1.2 ASH (%)

Ash content in tilapia fillets increased slightly at the end of the storage period of 120 days. Regarding ash it was observed that the treatment T2 recorded maximum (1.57%) ash, The treatment T0 recorded a minimum (1.27%) ash which was significantly inferior to the rest of treatments.

5.1.3 PROTEIN (%)

Protein content in tilapia fillets decreased slightly at the end of storage period of 120 days.It was observed from the data that the treatment T2 recorded maximum (18.48%) protein.The treatment T0 recorded a minimum (16.32%) protein which was significantly inferior to the rest of treatments.

5.1.4 NON-PROTEIN NITROGEN(%)

NPN content increased in tilapia fillets slightly at the end of the storage period of 120 days. It was observed from the data that the treatment T3 recorded maximum (0.54%) Non-Protein Nitrogen.The treatment T2 recorded a minimum (0.48%) Non-Protein Nitrogen which was significantly inferior to the rest of treatments.Thus, it was clear from the data that non-protein nitrogen varied with different treatments.

5.1.5 FAT(%) -

Fat content in tilapia fillets increased slightly at the end of the storage period of 120 days. Regarding fat it was observed from the data that the treatment T2 recorded maximum

(0.77%) fat. The treatment T3 recorded a minimum (0.65%) fat which was significantly inferior to the rest of treatments.

5.1.2. Effect of treatments on tilapia fillets during storage period in relation to physical analysis.

5.1.2.1 Drip Loss (%) -

Drip loss content in tilapia fillets increased significantly at the end of storage period of 120 days. It was observed from the data that the treatment T2 recorded maximum (7.68%) drip loss. The treatment T3 recorded a minimum (1.41%) drip loss which was significantly inferior to the rest of treatments.

5.1.2.2 L*, a* and b* colour value

Regarding the physical parameters of the tilapia fillets, it was observed that the mean L* value of colour of treatments varied from 76.51 to 82.04 among the treatments and mean of storage decreased towards the end of the from 81.94 at 0 day to 74.39 on 120 day. Thus, it was concluded that the lightness of fillets colour decreased with increase in the storage period. It was observed that the mean of a* value for colour of treatments varied from 2.61 to 4.82.During storage,mean a* value of colour decreased from 6.71 at 0 day to 0.77 on 120 day. It was noticed from the data that the mean b* value for fillets colour for treatments was in the range of 12.76 to 15.11. The mean b* value for fillets colour in storage decreased from 17.16 at 0 day to 9.64 on 120 day.

5.1.3. Effect of treatments on tilapia fillets during storage period in relation to biochemical Analysis.

5.1.3.1 TVBN (Total volatile basic Nitrogen) -

TVBN content in tilapia fillets increased significantly at the end of storage period of 120 days. It was observed from the data that the treatment T3 recorded maximum (17.25mg/100g) TVBN. The treatment T2 recorded a minimum (11.62mg/100g) which was significantly inferior to the rest of treatments.

5.1.4. Effect of treatments on tilapia fillets during storage period in relation to microbiological analysis.

TPC (Total plate count)

TPC content in tilapia fillets increased significantly at the end of storage period of 120 days.It was observed from the data that total plate count of the treatment T3 recorded a maximum (5.46 $\log_{10}$cfu/gm).The treatment T2 recorded a minimum (5.07 $\log_{10}$cfu/gm) which was significantly inferior to the rest of treatments.Thus, it was clear from the data that TPC varied with different treatments.

5.1.4.6 Sensory evaluation

The fillets stored at -18°C condition for 4 months showed different performances in sensory score. The fillets of T2 obtained the highest sensory score for colour (8.35) , flavour, texture as well as overall acceptability (8.60). The fillets T0 recorded the minimum score for different parameters

5.2 Conclusion

From the present investigation, titled "**STUDIES ON FREEZING AND STORAGE PERFORMANCE OF TILAPIA FILLETS**" could be concluded that different treatments to fillets prior to freezing in (T1), (T2) have an additional effect on storage performance in relation to proximate, biochemical, microbiological parameters on final (120) day of storage. Compared to treated fillets i.e. control (T0) showed significantly reduced storage performance in relation to proximate, biochemical, microbiological parameters. Even without pre-treatments IQF fillets (T0) showed better results than the air blast fillets (T3).

As regards to the organoleptic evaluation, fillets (T2) recorded the maximum sensory score for flavour, colour, odour and texture. Thus, the study suggests that the tilapia fillets should be given pre-treatment of NaCl + STPP + Blanching- Temp:75˚c, Time: 2 Min+ Glazing - 10% + IQF Freezing (Temp: -40˚C) & Frozen Storage (-18˚C) to improve their storage performance.

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APPENDIX - I

Temperature and relative humidity recorded at ambient temperature recorded at Standard Week under Killa, Roha conditions during the course of investigation (2021-2022)

Sr. No.	SMW	Ambient temperature conditions		
		Temperature(˚C)		Humidity (%)
		Max	Min	
1.	11/07/2022 to 17/07/2022	34.04	25.45	100
2.	18/07/2022 to 24/07/2022	32.84	24.65	91.4

3.	25/07/2022 to 31/07/2022	28.04	23.85	98.5
4.	01/08/2022 to 07/08/2022	31.66	24.42	100
5.	08/07/2022 to 14/08/2022	30.25	24.02	90.4
6.	15/08/2022 to 21/08/2022	32.40	24.55	93.2
7.	22/08/2022 to 28/08/2022	34.80	26.45	92.5
8.	29/08/2022 to 04/09/2022	32.40	23.21	100
9.	05/09/2022 to 11/09/2022	30.14	23.55	90.0
10.	12/09/2022 to 18/09/2022	31.35	23.64	93.1
11.	19/09/2022 to 25/09/2022	30.10	24.20	92.5
12.	26/09/2022 to 02/10/2022	30.02	25.15	100
13.	03/10/2022 to 09/10/2022	32.10	24.74	93.2
14.	10/10/2022 to 16/10/2022	30.45	23.50	92.0
15.	17/10/2022 to 23/10/2022	37.4	24.49	92.1

SMW: Standard Meteorological Week