# EFFECTS OF KOKUM JUICE (*Garcinia indica*) ON BIOCHEMICAL AND SENSORIAL QUALITY OF MARINATED MACKEREL FILLETS.

**BY**


**SAISHA SANDEEP CHOPDEKAR**

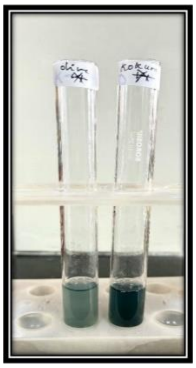
**SEAT NO- 22PO440027**

**ABC ID- 526-036-312-574**

**PRN-201902257**

**UNDER THE GUIDANCE OF ASSISTANT PROFESSOR IN ZOOLOGY-**

**MISS. GANDHITA VASANT KUNDAIKAR**


**SCHOOL OF BIOLOGICAL SCIENCE AND BIOTECHNOLOGY**

**ZOOLOGY PROGRAMME**

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# Effects Of Kokum Juice (*Garcinia indica*) On Biochemical and Sensorial Quality of Marinated Mackerel Fillets.

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by


**SAISHA SANDEEP CHOPDEKAR**

Seat no - 22PO440027

ABC ID- 526-036-312-574

PRN-201902257

Under The Supervision of Assistant Professor in Zoology-

**MISS. GANDHITA VASANT KUNDAIKAR**

School Of Biological Science and Biotechnology

Zoology Discipline



Goa University

April 2024


Examined by-

SBSB, M.Sc., Zoology, Taleigao Plateau, Goa 403206, GOA UNIVERSITY

Seal of the school-

# DECLARATION BY STUDENT-

I hereby declare that the data presented in this Dissertation report entitled, **"Effects of kokum juice *(Garcinia indica)* on biochemical and sensorial quality of marinated mackerel fillets"** is based on the results of investigations and analysis carried out by me in Zoology Discipline at the Goa University under the supervision/ mentorship of Ms. Gandhita Kundaikar and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations/ experimental or other findings given the dissertation. I hereby authorize the University authorities to upload this dissertation on the dissertation repository or anywhere else as the UGC regulations demand and make it available to any one as needed.

Miss. Saisha Chopdekar

Seat no- 22PO440027

Zoology Discipline

School of Biological Science and Biotechnology

Date-

Place- Goa University

# COMPLETION CERTIFICATE –

This is to certify that the dissertation report **"Effects of kokum juice (*Garcinia indica*) on biochemical and sensorial quality of marinated mackerel fillets"** is a bonified work carried out by **Ms. Saisha Sandeep Chopdekar** under my supervision in partial fulfilment of the requirements for the award of the degree of **Master of Science in Zoology** at the School of Biological Science and Biotechnology, Goa University.

Ms. Gandhita Kundaikar

Assistant Professor

Zoology

Date:

Dr. Bernard F. Rodrigues
Dean
School of Biological Science and Biotechnology
Date: 8-4-24
Place: Goa University

Dean of School of Biological Sciences
& Biotechnology
Goa University, Goa-403206

SBSB, M.Sc., Zoology * GOA UNIVERSITY *
Taleigao Plateau, Goa 403206

School Stamp

Date:

Place: Goa University

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# PREFACE

This thesis is submitted in fulfilment of the requirement of the Degree of Masters in Zoology. It comprises of research work carried out by the author under the supervision of Ms. Gandhita V. Kundaikar, Assistant Professor, Zoology Discipline, School of Biological Science and Biotechnology, Goa University from 2023 to 2024.

Fish is a high quality and inexpensive source of protein and contains important source of minerals. The demands for fish and its products are consumed in large numbers worldwide, however, being a highly perishable product, the quality of fish starts to deteriorate over time, therefore, to maximize the value of the fish its freshness quality must be maintained and to increase the acceptability of the fish product, the method of quality assessment must be known.

Different preservation techniques are needed to prevent fish spoilage and lengthen shelf life. They are designed to inhibit the activity of spoilage bacteria and the metabolic changes that result in the loss of fish quality (Teklemariam et al., 2015). Processing methods affect the basic components of meat, water content, protein, fats, minerals as well as sensory attributes such as aroma, texture, colour, smell which are based on biochemical reactions. The goal of fish processing and preservation is to deliver fish to the final consumer in a usable and good state (Karube et al., 2001).

Long term intake of synthetic antioxidants can cause health risk to humans (Sofia et al., 2019) therefore the importance of replacement of synthetic antioxidants with natural ones are highlighted in this thesis.

The thesis has been divided into four chapters. The first chapter is an introduction that gives information about different methods of preservation of fish and its demands, sensorial and biochemical assessment of fish quality, different fish species used, importance of fish processing and preservation, the fish species and the natural antioxidant selected for the present study, hypotheses as well as gaps and purposes along with the objectives and significance of the proposed work. The second chapter is a review of literature on the studies conducted on different fish processing and preservation techniques using different marinades. Chapter three includes information about sampling of the fish, preparation of chemicals and reagents, preparation of kokum juice, marination process and methods used for the analysis of biochemical compounds and sensorial analysis. The fourth chapter is focussing on analysis and conclusion that includes results and discussion where the observation and results obtained on different biochemical and sensorial tests is validated with other research work carried out earlier and is concluded with references.

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# ABBREVATIONS USED

| ENTITY | ABBREVATION |
|---|---|
| Thiobarbituric acid | TBA |
| Malondialdehyde | MDA |
| Kokum juice | KJ |
| Olive oil | OO |
| Hydroxyl citric acid | HCA |
| Butylated hydroxy anisole | BHA |
| Butylated hydroxytoluene | BHT |
| Sodium chloride | NaCl |
| Concentrated | Conc. |
| Sulphuric acid | $H_2SO_4$ |
| Optical density | OD |
| Nano meter | Nm |
| Perchloric acid | PCA |
| Milli litre | ml |
| Ferric chloride | $FeCl_3$ |
| Sodium hydroxide | NaOH |
| Standard | std |
| Trichloro acetic acid | TCA |
| Hydrochloric acid | HCL |
| Potassium hydroxide | KOH |
| Non- significant | NS |
| Figure | fig |

# ABSTRACT

Marinated mackerels are of economic importance in India. However, natural antioxidants affecting the quality of marinated are rarely addressed. The purpose of this study is to compare olive oil and kokum juice as fillers in mackerel marinades. The study investigates the effect of kokum juice on the quality of marinated mackerel fillets stored at $6^0$C for a period of 21 days. Mackerel fillets were marinated with 4% acetic acid and 12% NaCl, poured into glass jars, filled with either olive oil (control sample) or kokum juice (experimental sample) stored at $6^0$C. Changes in the sensory attributes were determined during the process and the following biochemical parameters were monitored: pH, thiobarbituric acid value, formaldehyde content, carbohydrate content, protein content, cholesterol content as well as amino acid content. Values of thiobarbituric acid and formaldehyde increased during storage whereas, pH, carbohydrates, protein, amino acid, and cholesterol decreased at the conclusion of storage period. Higher values of formaldehyde and cholesterol were found in olive oil samples than those with kokum juice samples. Samples treated with kokum juice demonstrated better biochemical and sensorial stability by scoring higher appearance, odour, flavour, colour, texture scores than olive oil samples.

Keywords: marination, mackerel fillets, kokum juice, olive oil, sensory, biochemical.

# CHAPTER 1.

# INTRODUCTION

## 1.1 FISH PROCESSING AND PRESERVATION

The goal of fish processing and preservation is to deliver fish to the final consumer in a usable and good state (Karube et al., 2001). As a result, paying special attention to minute details throughout the preparation, catching, landing, handling, storage, and transport processes is essential for providing a high-quality product (Gopakumar, 2000). People will become loyal clients if you regularly deliver high-quality products at a reasonable price (Nelson et al., 2004).

## 1.2 METHODS OF PRESERVATION OF FISH

Preservation can be done for both short and long periods (Eyo, 2002). Different methods of preservation include:

1.2.1 **CHILLING:** which is the first and simplest method to preserve and process fish (Tawari & Abowei, 2011) which is obtained by covering with layers of ice. Ice is not effective for long preservation, as melting water gets a sort of leaching of valuable flesh contents that are responsible for the flavours. Therefore, ice is effective only for short term preservation (FAO, 2007). Ice helps in reducing the growth rate of bacteria by decreasing the temperature of fish. Also, it washes away slime and bacteria as it melts (Idachaba, 2001).

1.2.2 **SALTING AND DRYING**: fish are dried and salted straight away in the sun.

1.2.3 **SMOKING:** simple method of preservation that imparts flavour to the food (Kauffeld et al., 2005).

1.2.4 **CANNING:** treatment of fish in sealed containers made of thin aluminium cans by fully sterilizing the product (Idachaba, 2001). It prevents contamination of unwanted pathogenic organisms by storing them in airtight package (Leistner & Gould, 2002).

1.2.5 **PICKLING:** various pickling methods are used worldwide for different fish species. Spices may be added.

## 1.3 DEMAND OF FISH PROCESSING AND PRESERVATION

Public interest in food quality and production has increased in recent decades, probably related to changes in eating habits and consumer behavior (Christensen et al., 2006). The demand for high quality and safety in food production calls for high standards for quality and process control which in turn requires appropriate analytical tools to investigate food. The main food properties contributing to total food quality and assessment include organoleptic and sensory attributes, food safety, nutritional value and functionality. The composition of proteins, essential amino acids, vitamins, minerals, carbohydrates, as well as non-nutrients with high biological activity (antioxidants) constitutes the nutritional value of the food products.

Fish and fish products cover an important part of protein demand of human nutrition and the total amount of consumed fish has dramatically increased in recent decades. There are several methods to assess the quality and freshness of fish and fish products such as physical, chemical, biochemical, microbiological, sensory analysis or tests.

The demand for fish processing in India has been steadily growing due to several factors such as market and size growth, nutritional awareness, advancement in aquaculture technologies, government initiatives, export markets, processing levels, food consumption trends. The worlds appetite for fish and fish product continues to grow. Goa being a coastal state, has a rich tradition of fishing and seafood consumption via various processing techniques. Marine and coastal fisheries contribute significantly to states economy, employment opportunities as well as food security.

## 1.4 SENSORY ASSESSMENT OF FISH AND FISH PRODUCTS

Odour, colour, texture, flavour, and juiciness are examples of sensory characteristics that can be used as helpful indicators of freshness and quality. Clear evaluation of fish quality standards requires the establishment of freshness or rotting indicators derived from precise procedures. One popular technique for figuring out and creating quality indices for significant sensory characteristics is sensory evaluation. It can be ascertained by various point-scoring schemes or systems. The majority of the time, the first chance to assess the quality of seafood is determined by its appearance. When determining the quality of fish, colour matters. The fish processing business uses sensory methods to assess the product's quality. It is possible to effectively identify all outward indications of deterioration through touch, smell, or sight. A number score sheet offers numerical measurements. The flavour and texture change occurs readily for a variety of reasons.

## 1.5 BIOCHEMICAL ASSESSMENT OF FISH AND FISH PRODUCTS

In biochemical assessments of quality, the various products of spoilage in fish muscle are quantitatively determined and correlated with sensory characteristics. These compounds are produced in fish muscle by autolytic enzymes or by chemical reactions like lipid oxidation. During spoilage, these compounds gradually accumulate in the flesh and hence their determination provides a measure of progress of spoilage. These compounds are thiobarbituric acid, formaldehyde content, ammonia concentration, peroxide value, etc.

## 1.6 FISH SPECIES USED IN FISH PROCESSING

Candidate fish species used for fish processing in India and worldwide include high value species (finfish and shell fish) such as groupers, mackerels, sardines, tuna, sardines, red snapper, seabass, tilapias, reef fishes, shrimps, crabs, lobsters, clams, oysters, mussels (FAO, 2007).

## 1.7 IMPORTANCE OF FISH PROCESSING AND PRESERVATION

Fish preservation and processing is a critical element of the fisheries. Fish farms and capturing locations are typically positioned distant from markets, posing risks of decomposition and uncertain market selling. When fish catch exceeds, it's important to preserve them for future use. Commercial fisheries rely heavily on preservation and processing methods. The fish are stored in a way that preserves their flavour, taste, odour, nutritional content, and digestibility for extended periods of time. To prevent waste of fish products, it's important to prioritize their preservation and processing. More research is needed on fish processing, which has received limited attention.

## **1.8 FISH SPECIES SELECTED -** Indian mackerel (*Rastrelliger kanagurta*)

## 1.8.1 CLASSIFICATION:

Kingdom- Animalia

Phylum- Chordata

Class- Actinopterygii

Order- Scombriformes

Family- Scombridae

Genus- Rastrelliger

Species- *R. kanagurta*

## 1.8.2 INDIAN MACKEREL (*Rastrelliger kanagurta*)

Fish is considered as one of the cheapest sources of protein, essential amino acid and unsaturated fatty acids in human diets. The proximate composition of any fish is good indicator of quality of fish (Sonavane et al., 2017). The Indian mackerel (Cuvier, 1817) is a small pelagic schooling scombroid fish widely distributed in the Indian Ocean and Indo-West Pacific region and is a very important species of commercial fisheries for the countries bordered with Indian Ocean, Indonesia, Pakistan, India, Sri Lanka, Bangladesh, Myanmar, Thailand (Collete & Nauen, 1983; Devaraj et al., 1997; AlMahdawi & Mehanna, 2010; Jayabalan et al., 2014). This species supports a rich commercial fishery a contribution to the total production (MFAA, 2013).

## 1.8.3 NUTRITIONAL COMPONENT OF INDIAN MACKEREL

Indian mackerel are commonly found on the Goan coast and are highly consumed by the people worldwide in various forms as it is a rich source of several essential nutrients such as omega-3 fatty acids, protein, vitamins B12 & D, and minerals such as selenium and phosphorus. Consuming Indian Mackerel fish regularly can help improve brain function, reduce inflammation, lower the risk of heart disease, boost overall health. It forms the major pelagic resources along the Indian coas (Yohannan & Saidkoya, 2000). As revealed from the studies made by different workers, Indian Mackerel is generally a plankton feeder (Chacko, 1949; Pradhan, 1956; Venkatraman, 1961; Noble, 1965). It has a high nutritional value and is considered a perishable food when its quality deteriorates during storage and/or during processing. Many consumers change their preference and acceptance towards fish products over time (Song et al., 2011)

The processing and preservation options of fish used during fish processing have a great impact on the sensory attributes and safety of the final product. Manufacturing procedures such as heat, pH, control of salt content are crucial to ensure the safety of the final fish product (Oxen & Knorr, 1993; Opstal et al., 2003; Höppner et al., 2004; Sevenich et al., 2013). However, cured fish products containing diverse amounts of preservatives (salt or vinegar) and thermally-processed products have different safety levels.

Fish muscle also contains vitamins, minerals and other nutritional compounds which are necessary in a diet (Larsen et al., 2007). Fish is not often consumed raw and frequently cooked and processed in several ways before consumption (Bognár, 1998). The significance of sea foods all over the world has gained attention because of high content of omega-3 and omega-6 fatty acids which have beneficial effects on the diseases such as cancer, inflammatory disease and coronary heart disease.

Indian Mackerel (*Rastrelliger kanagurta*) is popularly known as 'Bangada' and almost over 90% of the world's production of Indian Mackerel is contributed by India (Ayyappan, 2011). Although, many studies have examined the effect of different cooking conditions on the nutritional quality of fish, limited work has been done on Indian Mackerel.

Previous studies have shown that from the moment of capture, the spoilage process of mackerel begins (Martinsdóttir, 2010), as mackerel is fatty and perishable. With increasing consumer demand for fish and concern for seafood quality, especially in a market that has strict quality control, the price of mackerel is greatly influenced by sensory quality and consumers are willing to pay a higher price for high-quality fish (Sieffermann et al., 2013). Therefore, for monitoring changes in mackerel quality, diverse chemical and bacteriological analyses has been applied to the quality control of mackerel, such as Total volatile base nitrogen (TVBN) and trimethylamine (Postma et al., 2020; Agüeria et al., 2015; Calanche et al., 2019; Dos Santos et al., 2014; Lanzarin et al., 2016; Ritter et al., 2016), free fatty acids (FFAs) and peroxide values (PVs) (Okogeri & Chioma, 2016; Romotowska et al., 2017; Secci & Parisi, 2016), total viable counts (TVC) (Amaya et al., 2016; Jack & Read, 2008; Li et al., 2017; Sveinsdóttir et al., 2002), or specific spoilage organisms (SSOs) (Wu et al., 2019) and K-value (Mishima et al., 2005).

## 1.8.4 OTHER METHODS OF PRESERVATION

Some methods include ice coating or glazing, edible coating, addition of antioxidants, and vacuum packaging have been applied to mackerel products to extend the shelf-life of mackerel (Goulas & Kontominas, 2007; Jamróz et al., 2019; Quitral et al., 2009).

Antioxidants such as grape seed extract (GSE), papaya seed extract (PSE), sea weed extract (Fucus serratus and Polysiphonia fucoides) can restrain microbial growth and alleviate lipid oxidation and proteolysis (Babakhani et al., 2015; Sofi et al., 2016).

The richness and abundance of Indian Mackerel is restricted to Kutch, Karnataka and Goa coast and is a chief pelagic fishery along the south west coast of India and is a significant fish food and is frequently used in South and South-East Asian cuisine. It is a vital food fish that is consumed worldwide. The flesh of mackerel spoils quickly, particularly in the tropics, and is likely to cause scombroid food poisoning.

Accordingly, it should be eaten on the day of capture itself, unless properly cured or refrigerated. As it is a fatty fish, it has all the necessary protein, minerals and vitamins in the desired proportions. Omega-3 and Omega-6 fatty acids occur in high proportions in Indian Mackerel and comprises of vitamins A, B6, B12, C, D, E and various minerals also occur richly in the fish. (Patel & Mishra, 2017).

## 1.9 NATURAL ANTIOCIDANT SELECTED - Kokum (*Garcinia indica*)

### 1.9.1 CLASSIFICATION:

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Malpighiales

Family: Clusiaceae

Genus: Garcinia

Species: *G. indica*

### 1.9.2 KOKUM (*Garcinia indica*)

Kokum (*Garcinia indica*) is one of the important indigenous trees originated and grown in Western Ghats of India, South Konkan region of Maharashtra, Coorg, and Goa and is found in semi evergreen and evergreen forests and as a home garden tree (Chandran, 2005). Kokum belongs to the botanical family Clusiaceae and the fruit is usually spherical or globular in appearance which are green when raw and become red to dark purple when fully ripe with unique flavour.

Garcinia indica fruit rind shows the antioxidant, anticancer and anti-ulcer properties (Yamaguchi et al., 2000) which prevents infections and is used as a preservative.

### 1.9.3 NUTRITIONAL COMPONENTS OF KOKUM

It consists of a healthy mixture of ascorbic acid, manganese, potassium, dietary fiber, and garcinol along with vitamin C and B6. The potent antioxidant present in kokum is Garcinol.

Kokum contains other naturally occurring compounds with potential antioxidant properties such as citric acid, malic acid, polyphenols, carbohydrates, anthocyanin flavonoids and ascorbic acid (Cadenas & Packer, 1996; Peter, 2001; Rastogi & Nayak, 2010; Einbond et al*.,* 2004; Yoshikawa, 2000).

### 1.9.4 USES OF KOKUM

Kokum (*Garcinia Indica)* is an ancient fruit that is widely used for culinary, pharmaceutical, nutraceuticals and industrial uses and has a long history in Ayurvedic medicine as it was traditionally used to treat ear infection, sores, dysentery, dermatitis, diarrhea, and to facilitate digestion. The kokum fruit helps in fight cancer, paralysis and cholesterol. The kokum fruit is used to improve skin health. It also acts as a good digestive tonic.

The high-pressure performance liquid chromatography (HPLC) analysis of Garcinia indica fruit illustrates chief physiochemical hydroxyl citric acid (HCA) which is claimed to have fat-reducing properties in the human body. (S.B et al., 2014)

Kokum comprises three important chemical constituents or phytonutrients viz, Garcinol, Hydroxycitric acid and anthocyanin pigment are well known for their antioxidant, anti-inflammatory and anti-carcinogenic activity. Garcinol is a fat-soluble yellow pigment; Hydroxycitric acid is used as an acidulant and physiologically active compound has been revealed to significantly decrease body weight. (S.B et al., 2014)

Garcinol has been studied for its anti-cancer, anti-ulcer, anti-oxidative and antiglycation activity (Nayak et al., 2010) and is also reported to show some antimicrobial activity. (Chatterjee et al., 2003; Chatterjee et al., 2005).

Kokum fruits possess certain medicinal properties and juice extracted from this fruit is sweet and sour and thus liked by many thus, a glass of cold kokum syrup is refreshing and it also improves the digestive system. It is highly preferred by many people since it is a natural fruit extract.

It helps prevent infection as it has antifungal properties and also wards of aflatoxins thus used as a food preservative. It is a powerhouse of essential nutrients and vitamins in the body. Sundried kokum or kokum juice is often widely used as a souring agent as an additive in foods. Kokum is a plant which is used both as a medicine and as a spice.

The blackish red colour of kokum rind is due to the presence of antioxidant anthocyanins. Colour is an important constituent of any food as every food is identified and associated with certain colour. Colour is the first characteristic the consumer perceives of a food, which confers expectations of quality and flavour. All the products formulated with kokum powder and pulp has extremely attractive colour. Anthocyanin of kokum are water soluble. The overall acceptability of any product is the principal characteristic to ensure consumer acceptability of any product.

Consumer awareness and preference about health hazards associated with synthetic colours is increasing day by day and thus the use of natural colorants is growing. The use of natural pigments as food colorants permitted and legalized by the regulatory authorities is very inadequate and limited. Numerous studies assessed and evaluated different sources of anthocyanins along with the pigment concentration (Chetan et al., 2010) and the results specify and indicate that the kokum comprises highest concentration (i.e. 2400 mg/100g of fresh fruit) of anthocyanins as compared to other sources (Nayak et al., 2010) and is a rich source of natural pigments, which are water soluble and assist in scavenging free radicals. Hence, kokum can be more explored for its use as a natural colourant.

## 1.10 OLIVE OIL –

More sustainable food production is looked-for to meet and safeguard the food demands for future generations. Natural additives such as Flavoured Olive Oils (FOOs) have been proposed to ensure food quality and food safety through a more sustainable approach. Adding herbs and spices to olive oils is still an ancient and extensive practice and an object of research, considering the potential and budding application as preservative and flavouring agents. The bioactive compounds present in olive oil such as flavonoids, anthocyanin, phenols and carotenoids are responsible for anti-microbial, anti-inflammatory, anti-oxidant, anti-parasitic, anti-viral, anti-carcinogenic and anti-ulcerous properties including several beneficial effects on human health. The organoleptic properties of Flavoured Olive Oils (FOOs) is not always appreciated and cherished by consumers. (Trabelsi et al., 2021)

Fresh olive oil is becoming more and more popular among consumers, mainly because of the conviction that a Mediterranean diet can help prevent certain diseases (Boskou & Visioli, 2003, Paraskevopoulou et al., 2005). The diets of the nation's encircling the Mediterranean Sea depend heavily on olive oil.

Currently, plant extracts, spices, and essential oils are used in the food industry as "green" alternatives to synthetic additives for extending shelf life and for their effects against foodborne pathogens.

Given their potential use as flavouring and preservative agents, the age-old practice of blending herbs and spices with olive oils is still popular today and is the subject of research. A number of techniques have been proposed over the years to aromatize olive oil, including the infusion of natural material, the addition of essential oils, and the cold pressing of olives combined with spices, herbs, or vegetables. These techniques have varied effects on the flavoured oils' acceptability, oxidative stability, quality parameters, and presence of bioactive compounds.

The bioactive compounds (such as phenols, flavonoids, carotenoids, and anthocyanin.) usually present in the olive oil are accountable for antioxidant, antimicrobial, antiparasitic, antiviral, anti-inflammatory, antiulcerous, and anticarcinogenic properties, in addition to other several beneficial effects on human health.

## 1.11 OBJECTIVES

1. To evaluate proximate content of marinated fish fillets.

2. To assess fish’s freshness and shelf-life by sensory and biochemical methods.

## 1.12 HYPOTHESES

The incorporation of kokum juice in the marinade of mackerel fillets will dispense positive effects on the oxidative stability, chemical composition and sensory attributes of the resulting product. I foresee that the antioxidants incorporated in the kokum juice will help preserve the fillets by decelerating lipid oxidation and thus increasing their self-life. Furthermore, I speculate that kokum juice will impart unique flavors and colors to the marinated mackerel fillets, improving their overall sensory appeal.

Overall, the aim is to investigate the potential of kokum juice as a natural additive for enhancing the quality and consumer acceptability of marinated mackerel fillets through this study.

## 1.13 GAPS AND PUROPSES

### 1.13.1 Limited Understanding of the Sensory Attributes of Kokum-Infused Seafood:

**Gap:** The sensory aspects of marinated mackerel fillet with kokum juice have not been sufficiently investigated, with limited data on potential flavour and colour enhancements.

**Purpose:** To evaluate and document the sensory characteristics of mackerel fillets marinated with kokum juice infusions, providing valuable insights into consumer preferences and acceptance.

### 1.13.2 Potential for Natural Additives in Seafood Processing:

**Gap:** There is a growing interest in identifying natural additives for seafood processing to enhance the shelf life and acceptability, the limiting factor is the lack of comprehensive studies conducted for evaluating the applicability and effectiveness of kokum juice in this context.

**Purpose:** To investigate the effectiveness of kokum juice as a potential natural additive in improving the chemical and sensory qualities of marinated mackerel fillets, thereby contributing to sustainable and consumer-friendly seafood processing practices.

### 1.13.3 Integration of Traditional and Locally Available Ingredients in Modern Seafood Processing:

**Gap:** Readily available traditional ingredients like kokum are often underutilized in modern seafood processing, where synthetic additives are more prevalent.

**Purpose:** To look into the feasibility of integrating natural and traditional ingredients like kokum juice into modern seafood processing, aiming for a balance between traditional knowledge and modern processing techniques.

### 1.13.4 Limited knowledge of Kokum Juice in Seafood Marinades:

**Gap:** There is a scarcity of comprehensive studies that specifically look into the effects of kokum juice in the context of marinated mackerel fillets.

**Purpose:** To bridge this gap thorough analysis is to be carried out to evaluate how kokum juice influences the chemical, oxidative, and sensorial qualities of marinated mackerel fillets.

#### 1.13.5 Inadequate Understanding regarding the impact of Kokum Juice on Lipid Oxidation:

**Gap:** Diminutive research has been carried out in-depth to explore how kokum juice, with its antioxidant properties, may affect the oxidative stability of lipids in mackerel fillets.

**Purpose:** To elucidate how kokum juice can mitigate lipid oxidation, and thus improving the overall quality and shelf life of marinated mackerel fillet

## 1.14 SIGNIFICANCE OF THE PROPOSED WORK

There are several published studies indicating a relationship between the long-term intake of synthetic antioxidants and some health issues such as skin allergies, gastrointestinal tract problems and in some cases increased risk of cancer (Sofia et al., 2019).

High doses of synthetic antioxidants may cause DNA damage. BHA and BHT have already found to be responsible for adverse effects on liver and for carcinogenesis in animal studies.

The increasing suggestion of replacing synthetic antioxidants with natural ones is associated with benefits like enhancements of food quality, includes health interest, promotion of eco-friendly food system, processing more composite foods for maximum exploitation of natural antioxidants.

It is important to note that the replacement of synthetic antioxidants with natural ones is still a topic of research and debate. The implication of replacing synthetic antioxidants with natural ones in food systems are still being studied.

To the best of my knowledge, no work has been carried out on "Effects of kokum juice (*Garcinia indica*) on biochemical and sensorial quality of marinated mackerel fillets". Limited studies are present in the literature about marinating Indian Mackerel with natural indigenous antioxidant. This is the first study attempted in evaluating the biochemical and sensorial attributes of marinated Indian Mackerel with natural indigenous antioxidant stored at refrigerated conditions ($6^0$C) for a period of 21 days.

# CHAPTER 2.

# REVIEW OF LITERATURE

## 2.1 REVIEW OF LITERATURE

Fish is a very valuable food source due to good balance of all the essential nutrients like proteins, lipids, minerals and vitamins. But people are still unaware about its essential benefits and so they should be made aware by conducting various studies on the nutrients present in fish and assessing their health benefits (Balami et al., 2019).

A study was investigated on the shelf life of bonito and anchovy marinades stored at $4^0$C for a period of 170 days and biochemical, chemical and sensory quality were examined. Biochemical composition results showed that average crude protein, fat, moisture and ash rates decreased respectively over a period of time. The average pH values at the beginning and at the end of the storage were in defined limit values whereas the average values of TMA and POS of fresh and during the storage were under tolerable limits. The average TVB-N values decreased respectively. The result of sensory analysis was 130 days for marinated bonito and 155 days for marinated anchovy (Duyar & Eke, 2009).

Olgunoglu et al. (2009) evaluated sensory, chemical (TVB-N, TBA, peroxide values, free fatty acids, biogenic amines and pH) and microbial parameters of marinated anchovy fillets stored at $1+1^0$C and found that the concentration of TBA and PV were found to be more efficient than TVB-N in the determination of quality of marinated anchovy fillets. The histamine levels increased during storage period but did not exceed the toxic level as reported by the Food and Drugs Administration (FDA). Data obtained from this study show that the marinated fish samples can be stored for a period of 7 months at $1+1^0$C.

Gokoglu and Ucak (2020), investigated the effects of raw materials freshness on the quality of marinated fish which was divided into 2 batches kept at ambient temperature ($20^0$C) for 6 hours and $0^0$C for 72 hours into marination solution containing 3% acetic acid and 8% NaCl. TVB-N, TMA, TBA and para-anisidine values in both marinated samples increased significantly during storage at $4^0$C. The sensory scores of both samples decreased during storage but higher scores were obtained for samples kept at $0^0$C compared to samples kept at ambient temperature. The result of the study determines that the quality of the raw material affects the quality of marinated anchovy.

Another study was conducted to determine the effect of sea bream (*Sparus aurata*) marinated some quality properties during cold storage. The fillets were immersed into brine including 3.5% acetic acid for marination in sunflower oil and sauce prepared with sunflower oil. During storage, sensory, crude protein, lipid, dry matter, crude ash, TBA, TVB-N, TMA-N and peroxide analysis were done periodically. According to chemical and sensory analysis results obtained in the study, it was concluded that seabream marinades packaged as plain and sauced can be stored in $4^0$C for 200 days (Kaya & Basturk, 2015).

Study on effects of pomegranate sauce on the quality of marinated anchovy during storage at $4^0$C were investigated. Fillets were marinated with acetic acid, sunflower oil and pomegranate sauce. TVB-N, TMA-N values increased during storage. Higher values for free fatty acids (FFA), conjugated diens were found in samples with sunflower oil. Samples in pomegranate sauce showed better oxidative stability and produced desirable taste and flavour and was least effective as sunflower oil to keep quality (Gokoglu et al., 2009).

A study of shelf life of sardine marinades in tomato sauce was investigated. The results indicated that at the end of storage period of 6 months, the difference between TBA, FFA and pH value were not significantly different while the difference between TVB-N, TMA-N were significant (Kilinc & Cakli, 2005)

Improving the sensory quality of fish meat is crucial aspect to enhance fish consumption. In a study conducted by Mustafa Oz and Ucak (2023), on investigating quality parameters of common carp (*Cyprinus carp*) meat marinated using different ratios of lemon juice (0.00%, 40.00%, 50.00%, 60.00%) and salt (10.00%) at $18^0$C observed that lemon had positive effects on the shelf life of fish meat. Peroxide value, pH value, TBA value were lower in groups marinated with lemon juice and salt compared to control group. The panellists for sensory analysis liked the carp meat of the groups marinated with lemon juice.

In a study conducted in Turkey by Kaya et al. (2023) on comparative effect of olive oil and rosehip sauce on the quality of marinated anchovies stored at $4^0$C resulted that TVB-N, pH, peroxide value, TBA increased during storage. Higher values of TVB-N, TBA, peroxide value were found in samples with olive oil than with rosehip sauce. Rosehip sauce samples had higher appearance, odour, colour and texture scores than olive oil samples.

Sardine (*Sardina pilchardus*) fillets were marinated using different marinate solutions prepared with vinegar, grapefruit juice and pomegranate juice concentrate and were stored at $4^0$C for a period of 28 days. Sardines marinated with grapefruit juice had highest pH value at each evaluation period. On $28^{th}$ day, highest acid value was obtained in vinegar samples and lowest acid value was obtained in grapefruit sample. No significant difference in salt content were found between marinade treatments. Pomegranate samples has lowest peroxide and TBA value. The result concluded that pomegranate sauce could be used as a functional marinade solution in sardine marination (Serdaroglu et al., 2015).

The natural preservative effect of rosemary and thyme essential oils on marinated crayfish stored at $4^0$C was investigated. Microbiological results showed that natural compounds from rosemary and thyme essential oils resulted in lower bacterial growth during chilled storage period. TVB-N, TBA values increased in all groups. The shelf life of marinated crayfish was found to be 42 days, 56 days, 70 days according to the results of sensory and microbiological assessment (Duman et al., 2015).

A study was conducted on quality properties and shelf life of gibel carp (*Carassius gibelio*) marinades stored at $4^0$C in different sauces (Group A- sunflower oil and tomato paste, Group B- sunflower oil with garlic, red pepper, thyme, basil and mint, Control group- sunflower oil). Chemical analysis resulted TVB-N, TBA values increased in all groups during storage but did not exceed acceptable limit values. TVB-N and TBA values were highest in group A. at the end of 135 days, sensory analysis of Group B did not exceed acceptable limit values. The shelf life of sauced gibel carp marinades were 120 days (control), 105 days (group A), 135 days (group B) (Taskaya et al., 2016).

Another study was conducted on qualitative changes in anchovy fillets in a marinade made from fresh lemon juice and olive oil with the addition of acetic acid at $4^0$C and it required 7 days until sensory acceptability of the product was achieved. The lemon juice showed good preservative effect and reduced the bacterial count in the products while the addition of 0.5% acetic acid improved the sensory attributes of the products (Simat et al., 2019).

Another study described potential use of olive oil-lemon juice emulsions containing different levels of lemon juice (0, 25, 35, 50% v/v) as a flavouring, preservative and antioxidant agents in marinated anchovy. Increasing the levels of lemon juice in sauces retarded the chemical and oxidative changes of marinated anchovy. However, increasing the level of lemon juice more than 35% in sauces led to a decreased in sensorial acceptability. Chemical and oxidative qualities of all the sauced samples were in the limit of deterioration during 100 days of storage (Topuz et al., 2016).

Physical, chemical and microbiological analysis of the samples were performed to determine the effects of garlic and hot pepper sauce on shelf life of marinated anchovies. TVB, lactic acid bacteria count, biogenic amines, pH, TVB-N, TBA analysis were performed during 7 months of storage at $4^0$C. Comparing the control group with garlic added and hot pepper sauce added sample, garlic addition reduced the microbial growth and provided that the samples retained their physiochemical properties for a longer time period (Ficicilar et al., 2020).

In a study on the preservative properties of olive leaf extract in marinated anchovies conducted by Testa et al. (2019), determined that the extract improves their shelf life without modifying the organoleptic characteristics of the product which suggests that it could be considered in food industry as a natural antioxidant and antimicrobial food additive.

A study was carried out by Essid et al. (2020), on effects of pomegranate peel and artichoke leaf aqueous extracts on biochemical, microbiological and sensorial quality of sardine fillets for a period of 90 days. The antimicrobial activity of pomegranate peel and artichoke leaf extracts led to reduce total viable counts and showed better oxidative stability and higher content of polyunsaturated fatty acids. Higher values of free fatty acids and histamine were found in control samples whereas adding extracts of pomegranate peel and artichoke leaves significantly decreased TVB-N and TMA during storage. Greater colour and appearance scores were found in samples marinated with pomegranate peel and artichoke leaf extracts than control samples.

According to the study conducted by Korkmaz et al. (2021), on effect of sunflower oil in combination with pomegranate and plum sauce on chemical, microbial and sensorial properties of marinates carp (*Cyprinus carpio*) fillets. Sensory evaluation analysis showed that total appearance, odour, taste, texture scored increased during storage. TVB-N increased in all group after 1 month. Results showed that the shelf life of marinated carp fillets treated with sunflower oil and in combination with pomegranate and plum sauce was more than 1 month.

Trabelsi et al. (2021) studied marinated anchovies prepared with flavoured olive oils and fatty acid composition was assessed. In all samples, oleic acid was the most abundant fatty acid followed by linoleic acid and palmitic acid. The aromatization of the olive oil did not significantly influence the amount of individual fatty acids.

# CHAPTER 3.

# MATERIALS AND METHODOLGY

## 3.1 SAMPLING OF THE FISH

Fresh Indian Mackerel *(Rastrelliger kanagurta)* was purchased from local fish market, Panaji (Goa). The fish was directly transferred to laboratory within an hour after purchase. The fish were thawed in water. The temperature was water was controlled at $20^{0}$C. The head, bones and internal organs were removed and fillets were obtained. The fillets were washed under tap water and drained.

FIGURE: 3.1- Raw materials cleaned, washed, de-headed, de-gutted and fillets obtained.

## 3.2 CHEMICALS AND REAGENTS-

The chemicals used for the carrying out the estimations in this study were phosphate buffer, perchloric acid, chloroform, methanol, 1N sodium hydroxide, carbohydrate stock solution, anthrone reagent, distilled water, concentrated sulphuric acid, glycine stock solution, ninhydrin, ethanol, citrate buffer, hydrochloric acid, stannous chloride, cholesterol stock solution, glacial acetic acid, ferric chloride, orthophosphoric acid, sodium-potassium tartarate, sodium carbonate, copper sulphate, Folin's-Ciocalteau reagent, bovine serum albumin, tri-chloroacetic acid, Nash's reagent, malondialdehyde tetrabutyl ammonium salt, thiobarbituric acid stock solution.

The glass wares utilised for the purpose of estimations were test tubes, centrifuge tubes, micropipettes, measuring cylinder, test tube stand, glass rods, beakers and separating funnel.

Spectrophotometer, vortex, centrifuge and water bath were the instruments used for the estimation.

## 3.3 PREPARATION OF KOKUM JUICE-

Soak kokum in hot water for about 3 hours. Transfer into the blender and blend to paste. Transfer the paste into a bowl. Add required quantity of sugar and water to dilute the paste into thin liquid by mixing well. Boil for 15 minutes by stirring occasionally. Boil until the mixture thickens and form syrup consistency. Turn off the flame and add required quantity of salt. Add cumin powder if required and mix well. Pass the mixture through the sieve. Kokum syrup stays good for 2-3 months when refrigerated.

FIGURE: 3.3- Preparation of kokum juice.

## 3.4 MARINATION PROCESS-

Fish marination is one such fish products which consists of fresh, frozen, sundried or salted or portions of fish which are processed by treatment with a solution which includes plant extracts and acids like fruit juice, vinegar, wine and also includes spice, oil, sugar that are used to enhance the flavour, juiciness, tenderness and aroma of the fish products and adds on to extend its shelf life. Since ancient time period, the process of marination is carried out by using salt and acetic acid with a limited quality and shelf life which is used to find out the stability of the end product. Various salts like NaCl, $CaCl_2$ and acid like lemon juice, citric acid is used to increase the toughness of muscle with better texture of fish products during marination process. Marinades are usually semi-preserve acids added to the fish to retard the action of bacteria and enzymes, ensuing in a production of flavour and shelf life.

Marinating with plant extracts has the highest antioxidant effect and slow down the process of lipid oxidation.

Fish marinades are popular ready-to-eat seafood products worldwide. Due to its high nutritional value and no necessity of additional preparation by the consumer the consumption of marinades is constantly rising (Szymczak & Kolakowski, 2012). Fish marinades stored at cooler temperatures (4-6$^0$C) keep a long time. Marination process is also the best way to add on flavours to the fish. Indian Mackerel is the chief commercial fish used for marinated fish production and other common fish species such as herrings, anchovies, sardines etc.

Marination process was carried out according to the method of Gökoğlu et al., (2009) with a minor modification.

Acetic acid and NaCl were used for the marinade. Fish fillets were marinated using 4% acetic acid and 12% NaCl. The fillets were placed in glass jars and marinade solution was added till the fillets were fully immersed in a fish-fillet marinade solution. The fillets were kept in marinade solution for 72 hours at 6°C to complete the marination process. After the marination stage was completed, the fillets were removed from solution, filtered, and divided into two batches of 50 grams each and packed in sealed plastic containers. Acetic acid is the most frequently used base for the fish marination.

To the first batch -150 ml of olive oil was added and to the second batch- 150 ml of kokum juice was added.

The samples were stored at $6^0$C and were analysed on 0, $7^{th}$, $14^{th}$, $21^{th}$ days to determine changes in product quality.

FIGURE:3.4- Marination process using 4% acetic acid and 12% NaCl for 72 hours at $6^0$C.

FIGURE: 3.4.1- Marination process using 4% acetic acid and 12% NaCl for 72 hours at $6^0$C completed.


FIGURE: 3.4.2 - Marination process using kokum juice and olive oil at $6^0$C.

## 3.5 SENSORY ANALYSIS-

Sensory analysis of marinated mackerel fillets was performed by a panel of 6 trained panellist using a scoring test. Panellist consisted of personal and students of Institute of Hotel Management who had experience in evaluating seafood. Mackerel fillets were served to the panellist to evaluate the following sensory attributes: colour, flavour, juiciness, acid taste, odour and global appreciation.

Each parameter in the sensorial analysis were evaluated. The panellist has to judge on the basis of perceived intensity (1- low intensity, 5- high intensity).

The criterion for the selection of panellist was the experience in evaluating seafood. The panellist evaluated the samples for odour, appearance, taste and overall appearance on a 5-point hedonic scale (1= poor, 5= excellent) with the following quality scores: 5- indicated "very good quality", 4- indicated "good quality", 3- for acceptance and a score of 2-1 indicated "spoiled fish" (Kurtcan & Gonul, 1987)

The 5- point hedonic scale is the most widely used scale for measuring food acceptability. The consumers were asked to indicate their degree of liking of each sample on a 5-point hedonic scale, with neutral point at 3.

In this instance, the 5-point hedonic rating scale ranged from 1-"dislike extremely" to 5-"like extremely" in order to determine the likability and acceptance of the fish product.

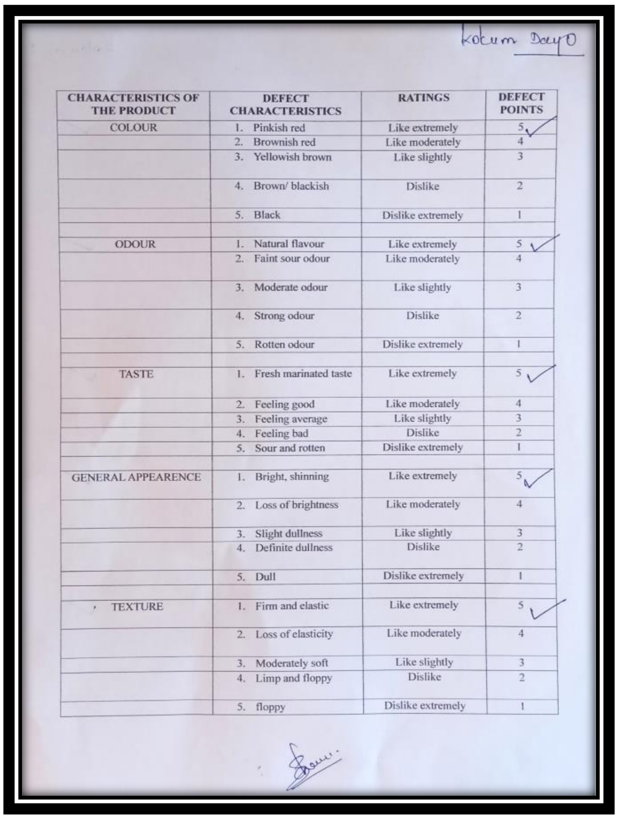
Appearance and colour were evaluated by visual assessing the product, then evaluated the aroma and finally the marinated mackerel fillets were tasted for texture and flavour liking. Taste intensities such as acidity, saltiness, sweetness, flavours such as fishiness, fatty/oil were examined.

5-point hedonic rating scale- ranging from

1= "not nearly enough"

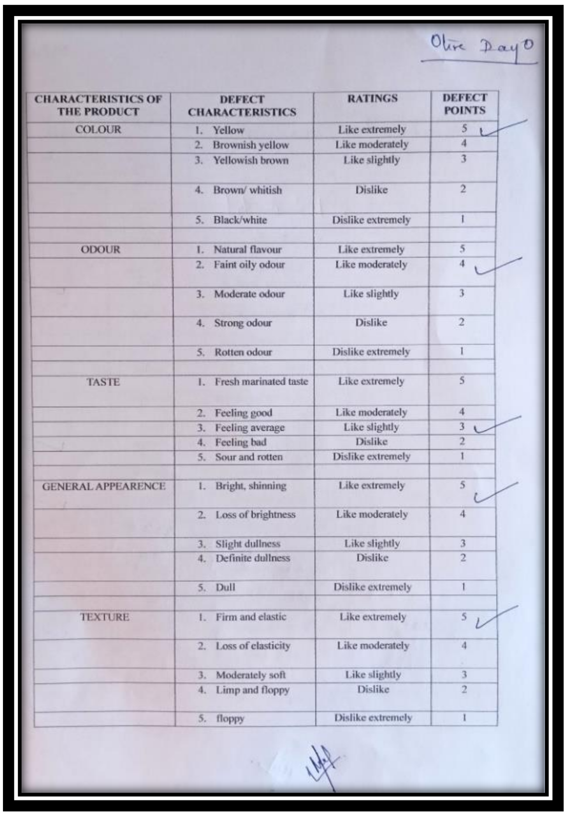
5= "too much"

3= "neutral point"



Kokum Day0

| CHARACTERISTICS OF THE PRODUCT | DEFECT CHARACTERISTICS | RATINGS | DEFECT POINTS |
|---|---|---|---|
| COLOUR | 1. Pinkish red | Like extremely | 5 ✓ |
| | 2. Brownish red | Like moderately | 4 |
| | 3. Yellowish brown | Like slightly | 3 |
| | 4. Brown/ blackish | Dislike | 2 |
| | 5. Black | Dislike extremely | 1 |
| ODOUR | 1. Natural flavour | Like extremely | 5 ✓ |
| | 2. Faint sour odour | Like moderately | 4 |
| | 3. Moderate odour | Like slightly | 3 |
| | 4. Strong odour | Dislike | 2 |
| | 5. Rotten odour | Dislike extremely | 1 |
| TASTE | 1. Fresh marinated taste | Like extremely | 5 ✓ |
| | 2. Feeling good | Like moderately | 4 |
| | 3. Feeling average | Like slightly | 3 |
| | 4. Feeling bad | Dislike | 2 |
| | 5. Sour and rotten | Dislike extremely | 1 |
| GENERAL APPEARENCE | 1. Bright, shinning | Like extremely | 5 ✓ |
| | 2. Loss of brightness | Like moderately | 4 |
| | 3. Slight dullness | Like slightly | 3 |
| | 4. Definite dullness | Dislike | 2 |
| | 5. Dull | Dislike extremely | 1 |
| TEXTURE | 1. Firm and elastic | Like extremely | 5 ✓ |
| | 2. Loss of elasticity | Like moderately | 4 |
| | 3. Moderately soft | Like slightly | 3 |
| | 4. Limp and floppy | Dislike | 2 |
| | 5. floppy | Dislike extremely | 1 |



Olive Day0

| CHARACTERISTICS OF THE PRODUCT | DEFECT CHARACTERISTICS | RATINGS | DEFECT POINTS |
|---|---|---|---|
| COLOUR | 1. Yellow | Like extremely | 5 ✓ |
| | 2. Brownish yellow | Like moderately | 4 |
| | 3. Yellowish brown | Like slightly | 3 |
| | 4. Brown/ whitish | Dislike | 2 |
| | 5. Black/white | Dislike extremely | 1 |
| ODOUR | 1. Natural flavour | Like extremely | 5 |
| | 2. Faint oily odour | Like moderately | 4 ✓ |
| | 3. Moderate odour | Like slightly | 3 |
| | 4. Strong odour | Dislike | 2 |
| | 5. Rotten odour | Dislike extremely | 1 |
| TASTE | 1. Fresh marinated taste | Like extremely | 5 |
| | 2. Feeling good | Like moderately | 4 |
| | 3. Feeling average | Like slightly | 3 ✓ |
| | 4. Feeling bad | Dislike | 2 |
| | 5. Sour and rotten | Dislike extremely | 1 |
| GENERAL APPEARENCE | 1. Bright, shinning | Like extremely | 5 ✓ |
| | 2. Loss of brightness | Like moderately | 4 |
| | 3. Slight dullness | Like slightly | 3 |
| | 4. Definite dullness | Dislike | 2 |
| | 5. Dull | Dislike extremely | 1 |
| TEXTURE | 1. Firm and elastic | Like extremely | 5 ✓ |
| | 2. Loss of elasticity | Like moderately | 4 |
| | 3. Moderately soft | Like slightly | 3 |
| | 4. Limp and floppy | Dislike | 2 |
| | 5. floppy | Dislike extremely | 1 |

Figure 3.5- Sensory analysis of mackerel fillets marinated with kokum juice and olive oil using 5-point hedonic scale.

## 3.6 METHODOLOGY-

**3.6.1 EXTRACTION AND ESTIMATION OF CARBOHYDRATES-** by anthrone test (Hedge & Hofrreiter, 1962)

PRINCIPLE- anthrone test is a test used for detection of carbohydrates. On addition of anthrone, carbohydrates get dehydrated with reaction with conc. sulphuric acid to form furfural. Furfural further reacts with anthrone to give a bluish green complex that can be quantified spectrophotometrically at 620nm.

PREPARATION OF REAGENTS-

1. Anthrone reagent - 0.2g anthrone powder in 100ml conc.$H_2SO_4$.
2. Glucose stock solution – 5mg of glucose in 500ml distilled water.

EXTRACTION-

The fish samples were cleaned and then dissected. About 5gms of the muscle tissue was extracted which was homogenised with 10ml of phosphate buffer. The homogenate was stored in blue capped centrifuge tubes and 1/3$^{rd}$ of it was used for the estimation of carbohydrates.

ESTIMATION

Standard solution of glucose was added in a serially increasing manner in 8 test tubes (0.1-1ml). This was diluted up to 1ml by using distilled water. The blank test tube contained just 1ml of distilled water. After this 4 ml of anthrone reagent was added to test tubes and then they were kept in a boiling water bath for about 15 minutes. The OD was then read at 620 nm. For the sample, 1ml of the extracted fish sample was taken and then 4 ml of anthrone reagent was added. This was followed by incubation in a boiling water bath for 15 minutes and finally absorbance was read against the blank at 620 nm.

### 3.6.2 ESTIMATION OF TOTAL FREE AMINO ACIDS BY NINHYDRIN METHOD

(Mahesha, 2012)-

PRINCIPLE- in the pH range of 4-8, all the α- amino acids react with ninhydrin, a powerful oxidising agent to give a purple-coloured product called Rhuimann's purple which can be measured calorimetrically at 570nm.

PREPARATION OF REAGENTS-

Ninhydrin solution: 1g of ninhydrin powder was mixed with 25ml of ethanol and 40mg of stannous chloride was mixed with 25ml of citrate buffer. Both the solutions were mixed to form the ninhydrin solution.

Citrate buffer: 0.21 g of citric acid and 0.29 g of sodium citrate were dissolved in 10ml of distilled water separately. 7.6ml of citric acid and 7.4ml of sodium citrate was mixed then the volume was made to 30ml with distilled water. The pH was then adjusted to 5.

5% Perchloric acid - 3.6ml PCA was mixed with 46.4 ml distilled water

Glycine stock solution was prepared by mixing 50mg of glycine powder in 50ml of 5% PCA.

EXTRACTION

The fish samples were cleaned and then dissected. About 5gms of the muscle tissue was extracted which was homogenised with 10ml of phosphate buffer. Equal amount of perchloric acid was added which was then centrifuged at 4000 rpm for 10 minutes. The supernatant thus obtained was used for estimation of amino acids.

ESTIMATION

The standard amino acid solution was pipetted in 8 different test tubes in a serially increasing manner. 1ml distilled water was added in the blank test tube. For the unknown test tube, 0.1ml of the fish sample extract was taken. Then, to all the test tubes, add 1N NaOH to make up the volume and add 2 ml of ninhydrin was added. Followed by this the tubes, were incubated in the boiling water bath for 15 minutes. After the incubation 2.5ml of 50% ethanol was added in all the test tubes and then the tubes were incubated at room temperature for 15 minutes. Finally, the absorbance was taken against the blank at 575 nm.

**3.6.3 ESTIMATION OF CHOLESTROL** (Zak & Ressler, 1955)

PRINCIPLE- cholesterol gives a brown colour in the presence of ferric chloride, acetic acid and concentrated $H_2SO_4$. The colour developed is due to dehydration of cholesterol to form 5-cholesterol-3- diene, which is oxidised by $H_2SO_4$ and polymerase to form a dimer/ trimer. The cholesterol and its polymer react with $H_2SO_4$ to form mono and di-sulphuric acid which are highly coloured in the presence of metal ion like ferrous.

PREPARATION OF REAGENTS

$FeCl_3$ – Acid mixture – 0.050g of $FeCl_3$ powder was dissolved in 20ml orthophosphoric acid and this mixture was then added in 50 ml of concentrated sulphuric acid.

Cholesterol stock- 0.010g of cholesterol powder was in 40ml acetic acid.

EXTRACTION

The fish samples were cleaned and then dissected and about 5gms of the muscle tissue was extracted which was homogenised with 10ml of phosphate buffer. Equal amount of perchloric acid was added to remaining homogenate. This was then centrifuged at 4000 rpm for 10 minutes. The supernatant thus obtained was separated. After this to the remaining residue of the muscle tissue about 4ml of chloroform and 2ml of methanol was added. This mixture was then again centrifuged at 4000 rpm for 10 minutes. The supernatant obtained hence was used for cholesterol estimation.

ESTIMATION

Cholesterol standard solution was added in a serially increasing manner in 8 test tubes. A blank was also maintained containing 3ml acetic acid instead of the standard. The volume in the tubes was made up to 3ml using acetic acid. Then about 1ml of ferric chloride was added in each of the test tubes and they were incubated at room temperature for 15 minutes followed by which the optical density was measured by using a spectrophotometer at 550 nm. For the sample, the cholesterol extract obtained was used in the required quantity and then its volume was made up to 3 by using acetic acid. Then 1ml of ferric chloride solution was added and there after incubation was done at room temperature for 15 minutes. Finally, the OD was read at 550 nm.

**3.6.4 ESTIMATION OF PROTEINS-**(Lowry et al., 1951)

PRINCIPLE- The principle behind the lowry's method of determining protein concentration lies in the reactivity of nitrogen with copper (II) ions under alkaline conditions and the subsequent reduction of Follin's reagent to heteropolymolypdenum blue by the copper catalysed oxidation of aromatic acids. Blue colour develops due to phenolic group of tyrosine and tryptophan.

PREPARATION OF REAGENT

1. Lowry's reagent

a. Solution A - 4g of sodium carbonate was added in 100 ml of distilled water.

b. Solution B - 0.1g of copper sulphate was added in 5ml of distilled water.

c. Solution C – 0.2g of Sodium-Potassium Tartrate was added in 5 ml of distilled water

98 ml of solution A + 1ml of solution B + 1ml of solution C was mixed together to prepare the Lowry's reagent.

2. Folin's Ciocalteau reagent 10 ml of Folin's reagent was added in 10 ml of distilled water to make 1:1 Folin's solution.

3. BSA solution was prepared by dissolving 10mg bovine serum albumin in 40ml 1N NaOH.

EXTRACTION

The fish samples were cleaned and then dissected and about 5gms of the muscle tissue was extracted which was homogenised with 10ml of phosphate buffer. Equal amount of perchloric acid was added to remaining homogenate. This was then centrifuged at 4000 rpm for 10 minutes. The supernatant thus obtained was separated. After this to the remaining residue of the muscle tissue about 4ml of chloroform and 2ml of methanol was added. This mixture was then again centrifuged at 4000 rpm for 10 minutes. The supernatant obtained hence was discarded. After this again a volume of 5ml of perchloric acid was added to the remaining muscle tissue residue and centrifugation was done at 4000 rom for 10 minutes. The supernatant obtained was discarded. After this 2ml of 1N NaOH was added to the remaining residue and this was kept in a boiling water bath for half an hour to get a clear solution. This clear solution was then used for the estimation of proteins.

ESTIMATION

The BSA standard solution was added in serially increasing concentrations in 8 of the test tubes. This was then diluted up to distilled water. Then 5ml of Lowry's reagent was added and incubation was done for up to 10 minutes. This was followed by the addition of 0.5ml of Folin's reagent and again incubation for 10 minutes. Finally, the absorbance was read against the blank at 660 nm. For the sample, 0.1ml of the extracted fish tissue sample was taken and in that 5ml of Lowry's reagent was added and this was followed by incubation for 10 minutes. Then 0.5ml of Folin's reagent was added and again incubation was done for about 10 minutes. The absorbance was then finally read at 660 nm. Concentration of the proteins was determined from the unknown OD by using the standard curve.

**3.6.5 DETERMINATION OF pH VALUE (ISO 11289: 1993 (E)-** (approved by Scientific panel on methods of sampling and analysis)

For pH measurements, the fish was homogenised with distilled water 1:2 (w/v) and measured with calibrated pH meter.

The pH value was determined by dipping a pH electrode into homogenates of filleted muscle in distilled water (1/1) (Manthey et al. 1988). All measurements were performed at room temperature using pH-meter.

PRINCIPLE- pH is the measurement of $H^+$ ion activity. It measures active acidity. pH may be determined by measuring the electrode potential between glass and reference electrodes; pH meter is standardized using standard pH buffers.

METHOD OF ANALYSIS-

After the sample preparation was done; i.e. homogenization of fish fillet sample with distilled water, immerse or embed the electrode and ensure that there is adequate contact between the probe and sample. Readings were noted when the meter reading was stable.

**<u>3.6.6 DETERMINATION OF FORMALDEHYDE-</u>** (Jaman et al., 2015)

<u>PRINCIPLE-</u> Formaldehyde reacts quickly with muscle tissue in fish causing protein denaturation. The formaldehyde reacts with an ammonium salt and acetylacetone under neutral conditions to form a coloured compound. The colour intensity of the formed compound is measured using a spectrophotometer at a specific wavelength at 415 nm. The absorbance is directly proportional to the formaldehyde concentration.

<u>PREPARATION OF REAGENTS-</u>

Nash reagent- was prepared by mixing 2ml of acetic acid and 2ml of acetyl acetone.

6% trichloroacetic acid- was prepared using 3.6g of TCA in 60ml distilled water.

<u>METHOD OF ANALYSIS-</u>

The fish samples were cut into small pieces. Then fish flesh was taken into blender for homogenization and blended for 10 minutes. Then a 60 ml of 6% tri-chloro-acetic acid was added for extraction of formaldehyde from the fish flesh. The extracted solution was then filtered by a Whatman No.1 of filter paper. Then pH of the solution was determined by a pH meter. Though the addition of tri-chloro-acetic acid reduced the pH value of the sample it was adjusted the pH between 6-7 of the sample by using Potassium hydroxide (KOH) and Hydrochloric acid (HCl).

Then 5 ml of sample solution was taken in a 50 ml of volumetric flask. Then the sample was kept in a freeze (- 200 C) for 1 h.

During analysis, the sample was taken out of the freeze and 2 ml of previously prepared Nash's reagent was added as indicator.

Fish sample was then heated in the water bath at 100 C for 30 minutes. The absorbance of the sample in cuvette was measured at 415 nm immediately by UV/v spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Triplicate of the absorbance was made for each sample and recorded for further calculation. The sample reading was placed in the standard curve for the calculation of formaldehyde content of the sample.

**3.6.7 ESTIMATION OF THIOBARBITURIC ACID- (Alam Zeb & Fareed Ullah, 2016).**

PRINCIPLE- MDA (malondialdehyde) serves as a marker for lipid peroxidation that reacts with thiobarbituric acid in glacial acetic acid medium. The reaction forms a coloured complex that can be detected spectrophotometrically. The absorbance is measured at 532 nm.

PREPARATION OF REAGENTS-

MDA standard solution- 31.35mg of MDA (malondialdehyde tetrabutyl ammonium salt) was dissolved in 100ml of glacial acetic acid.

TBA solution- 57.66mg of TBA was dissolved in glacial acetic acid (100 ml).

ESTIMATION-

The standard MDA solution was pipetted in 8 different test tubes in a serially increasing manner. 1ml TBA was added in the blank test tube. For the unknown test tube, 1ml of the fish sample extract was taken. Followed by this the tubes, were incubated in the boiling water bath for 60 minutes. Finally, the absorbance was taken against the blank at 532 nm.

# CHAPTER 4.

# ANALYSIS AND CONCLUSIONS

## 4.1 RESULT

Biochemical analysis of the mackerel fillets marinated with olive oil and kokum juice during the storage period of 21 days.

| | DAY 0 | | DAY 7 | | DAY 14 | | DAY 21 | |
|---|---|---|---|---|---|---|---|---|
| | OO | KJ | OO | KJ | OO | KJ | OO | KJ |
| PH | 4 ± 0.01 | 2.63 ± 0.02 | 4.01 ± 0.01 | 2.62 ± 0.02 | 4.05 ± 0.01 | 2.61 ± 0.01 | 4.09 ± 0.00 | 2.60 ± 0.00 |
| CARBOHYDRATES | 0.033 ± 0.00 | 0.083 ± 0.00 | 0.0189 ± 0.00 | 0.072 ± 0.00 | 0.0185 ± 0.00 | 0.062 ±0.00 | 0.031 ± 0.00 | 0.062 ± 0.00 |
| AMINO ACIDS | 80.638 ± 1.35 | 120.21 ± 1.20 | 26.81 ± 1.19 | 48.72 ± 1.20 | 7.97 ± 1.05 | 22.65 ± 1.35 | 6.27 ± 1.05 | 20.21 ± 1.20 |
| CHOLESTROL | 0.743 ± 0.07 | 2.50 ± 0.09 | 0.79 ± 0.04 | 2.33 ± 0.04 | 0.93 ± 0.05 | 2.214 ± 0.08 | 2.21 ± 0.10 | 1.00 ± 0.04 |
| PROTEINS | 1213.3 ± 8.27 | 1645.8 ± 10.61 | 1089.6 ± 8.89 | 1028 ± 9.90 | 956.6 ± 7.07 | 780.8 ± 8.20 | 733.1 ± 7.28 | 540.8 ± 8.20 |
| FORMALDEHYDE | 12.40 ± 0.84 | 20.62 ± 0.89 | 14.36 ± 0.50 | 20.90 ± 1.02 | 15.54 ± 0.90 | 21.99 ± 0.77 | 16.54 ± 1.54 | 23.36 ± 0.90 |
| THIOBARBITURIC ACID | 0.60 ± 0.01 | 1.72 ± 0.01 | 0.62 ± 0.01 | 1.76 ± 0.01 | 0.63 ± 0.01 | 1.77 ± 0.01 | 0.66 ± 0.02 | 1.82 ± 0.01 |

Table 4.1: Changes in Biochemical analysis for samples treated with olive oil and kokum juice during the storage period.

(OO- olive oil treated samples, KJ- kokum juice treated samples)

| | OLIVE OIL | KOKUM JUICE | P VALUE | * |
|---|---|---|---|---|
| PH | 4.04 ± 0.04 | 2.62 ± 0.01 | <0.0001 | **** |
| CARBOHYDRATES | 0.026 ± 0.08 | 0.070 ± 0.010 | 0.0030 | ** |
| AMINO ACIDS | 30.67 ± 35.20 | 52.74 ± 46.25 | 0.0282 | * |
| CHOLESTROL | 1.17 ± 0.70 | 2.01 ± 0.68 | 0.3103 | NS |
| PROTEINS | 996.7 ± 203.4 | 998.8 ± 474.9 | 0.9894 | NS |
| FORMALDEHYDE | 21.72 ± 1.24 | 14.71 ± 1.78 | 0.0004 | *** |
| THIOBARBITURIC ACID | 0.63 ± 0.03 | 1.8 ± 0.0 | <0.0001 | **** |

Table 4.1.2 : Data subjected to analysis of variance (ANOVA) to find the biochemical effect of olive oil and kokum juice on marinated mackerel fillets.

| | P VALUE (ANOVA) | * | P VALUE (FRIEDMANS TEST) | * |
|---|---|---|---|---|
| PH | 0.8348 | NS | >0.9999 | NS |
| CARBOHYDRATES | 0.2604 | NS | 0.1667 | NS |
| AMINO ACIDS | 0.0041 | ** | 0.0417 | * |
| CHOLESTROL | 0.9999 | NS | >0.9999 | NS |
| PROTEINS | 0.1077 | NS | 0.0417 | * |
| FORMALDEHYDE | 0.0326 | * | 0.0417 | * |
| THIOBARBITURIC ACID | 0.0218 | * | 0.0417 | * |

Table 4.1.3: Data subjected to analysis of variance (ANOVA) and Friedmans non-parametric test to find the day-wise biochemical effect of olive oil and kokum juice on marinated mackerel fillets during the storage period (Day 0-Day 21).

| | PAIRED T TEST |
|---|---|
| PH | p <0.0001<br>**** |
| CARBOHYDRATES | p = 0.0030<br>** |
| AMINO ACIDS | p = 0.0282<br>* |
| CHOLESTROL | p = 0.3103<br>NS |
| PROTEINS | p = 0.9894<br>NS |
| FORMALDEHYDE | p = 0.0004<br>*** |
| THIOBARBITURIC ACID | p <0.0001<br>**** |

Table 4.1.4 : Data subjected to paired T test to compare the means of the biochemical effect of olive oil and kokum juice on marinated mackerel fillets from day 0 to day 21.

| | ONE SAMPLE T TEST | |
|---|---|---|
| | OLIVE | KOKUM |
| PH | p<0.0001 **** | p <0.0001 **** |
| CARBOHYDRATES | p=0.0077 ** | p=0.0008 *** |
| AMINO ACIDS | p= 0.1799 NS | p= 0.1069 NS |
| CHOLESTROL | p= 0.0443 * | p= 0.0097 ** |
| PROTEINS | p= 0.0023 ** | p= 0.0245 * |
| FORMALDEHYDE | p<0.0001 **** | p=0.0005 *** |
| THIOBARBITURIC ACID | p <0.0001 **** | p<0.0001 **** |

Table 4.1.5 : Data subjected to One Sample T test to compare the means of the biochemical effect of olive oil and kokum juice on marinated mackerel fillets.

4.1.1 Estimation of pH:

At the end of 21 days, when the pH value was compared between control sample (mackerel fillets treated with olive oil) and experimental sample (mackerel fillets treated with kokum juice), it was determined to be highly significant, ($p < 0.0001$).

Since $p > 0.05$, in both the samples i.e. control sample as well as experimental sample when compared day-wise, it does not indicate any significant variation across the study period of 21 days.

In olive oil-treated samples, pH concentration gradually increased while the pH concentration in kokum juice-treated samples decreased over the course of storage period.

Both the samples treated with marinades were found to be acidic in nature.

The decline and rise in of pH levels was observed in Figure 4.1.1 (B).

pH value was comparatively higher in olive oil treated mackerel fillets than kokum juice treated mackerel fillets.

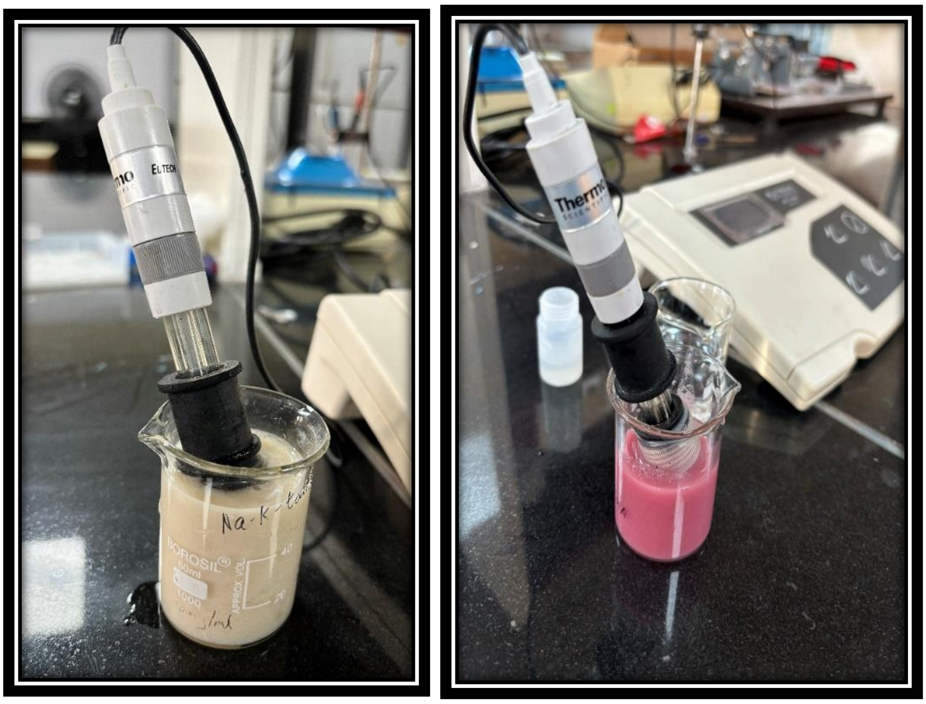


FIGURE 4.1.1 - DETERMINATION OF pH USING pH METER

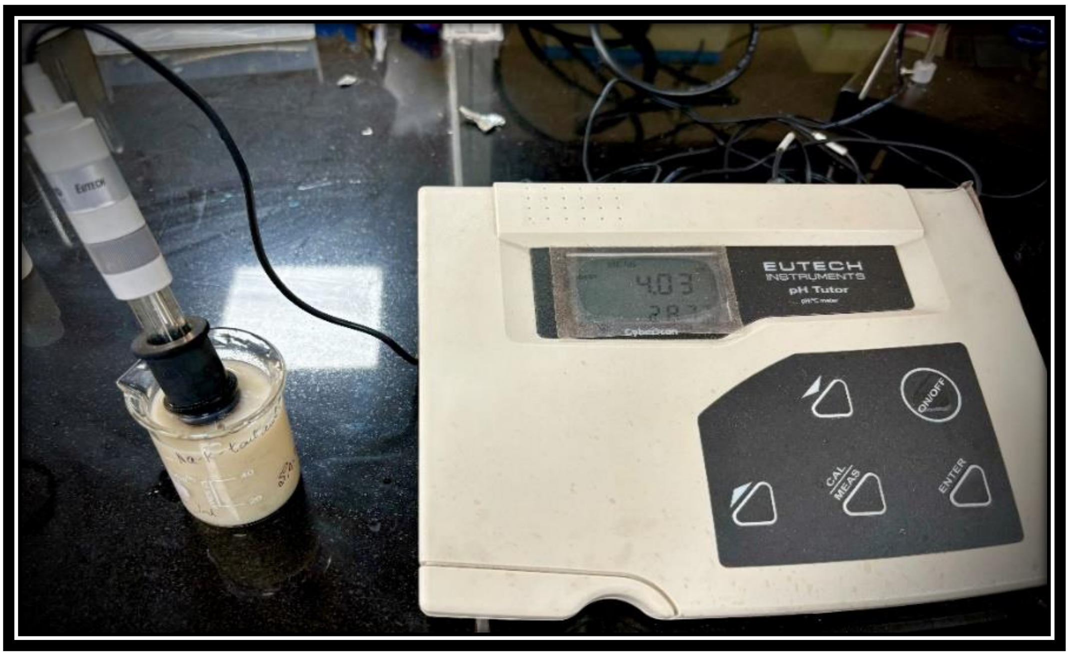




FIGURE 4.1.1(A) - DETERMINATION OF pH USING pH METER.

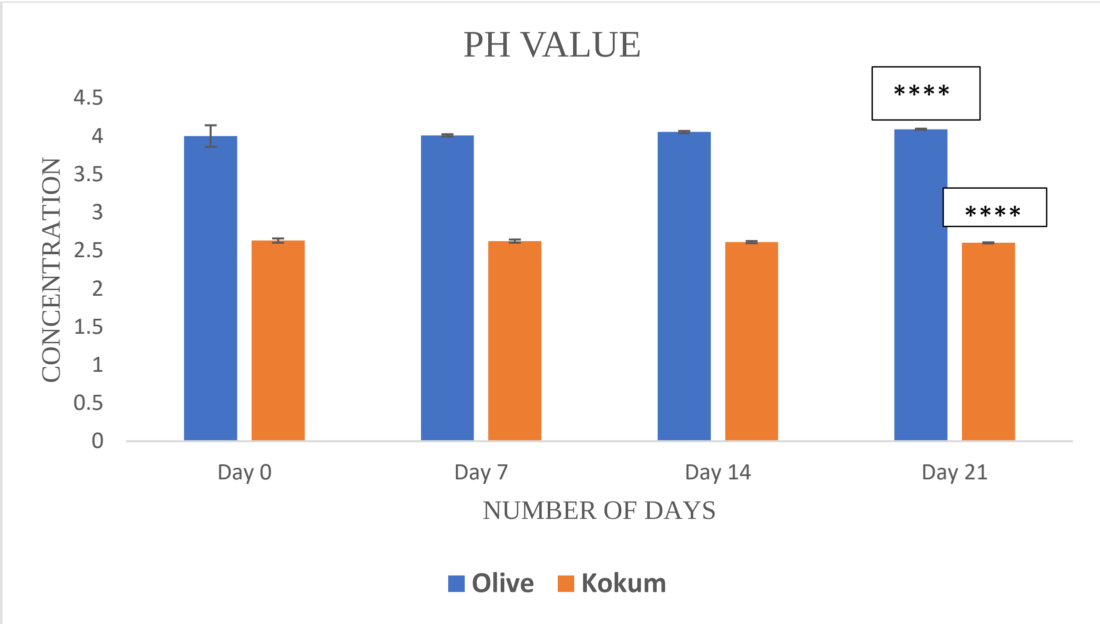



Figure 4.1.1 (B): Bar graph observed for the concentration of pH during the storage period.

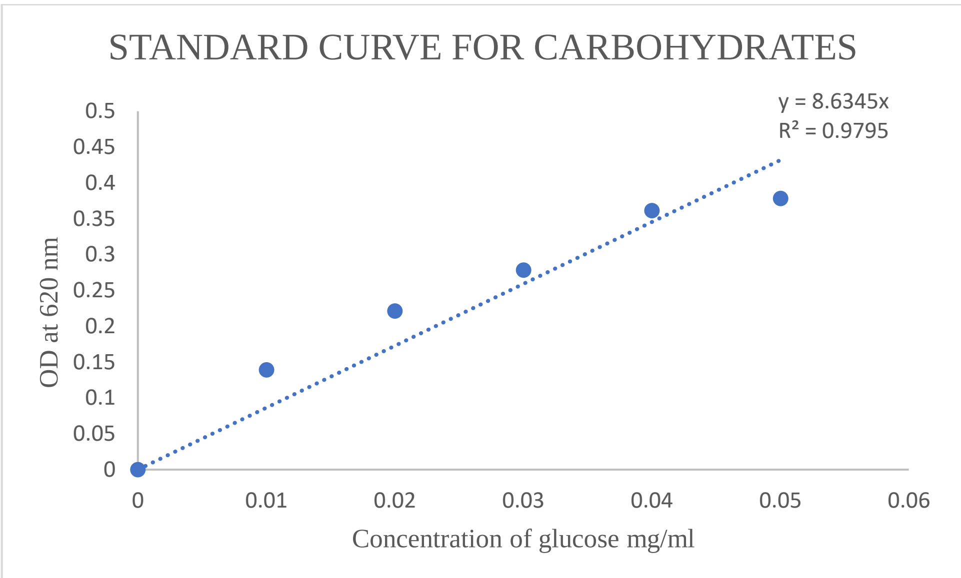
4.1.2 Estimation of Carbohydrates:

When the carbohydrate concentration was compared between control sample (mackerel fillets treated with olive oil) and experimental sample (mackerel fillets treated with kokum juice), definite significant difference, $p<0.05$ was observed at the end of storage period.

When compared day-wise, since $p>0.05$, there was no clear significant difference in the carbohydrate concentration across the days studied.

As seen in Figure 4.1.2 (B), the concentration of carbohydrates in the samples treated with kokum juice and olive oil decreased gradually throughout the course of the storage period.

The samples treated with olive oil had a relatively larger concentration of carbohydrates than the samples treated with kokum juice.




4.1.2 STANDARD CURVE FOR CARBOHYDRATES

DAY 0

DAY 7


DAY 14


DAY 21

FIGURE: 4.1.2(A) - ESTIMATION OF CARBOHYDRATES

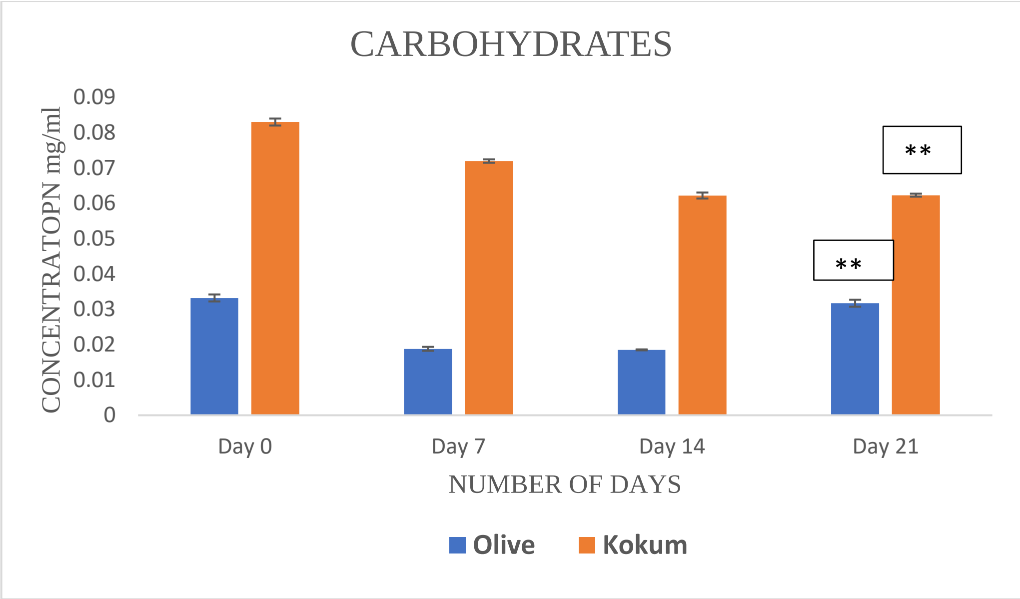



Figure 4.1.2 (B): Bar graph observed for the concentration of carbohydrates during the storage period.

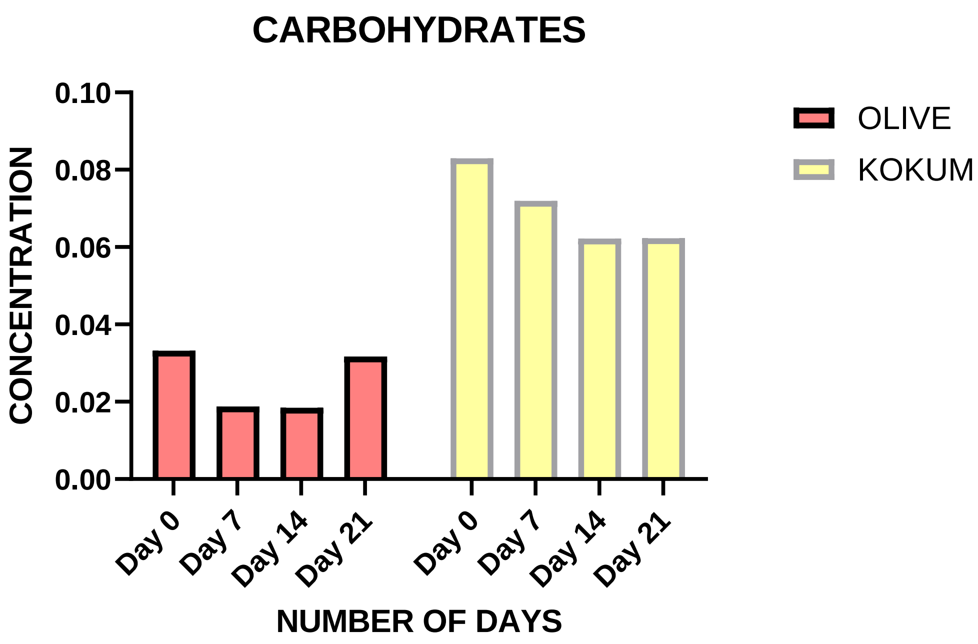


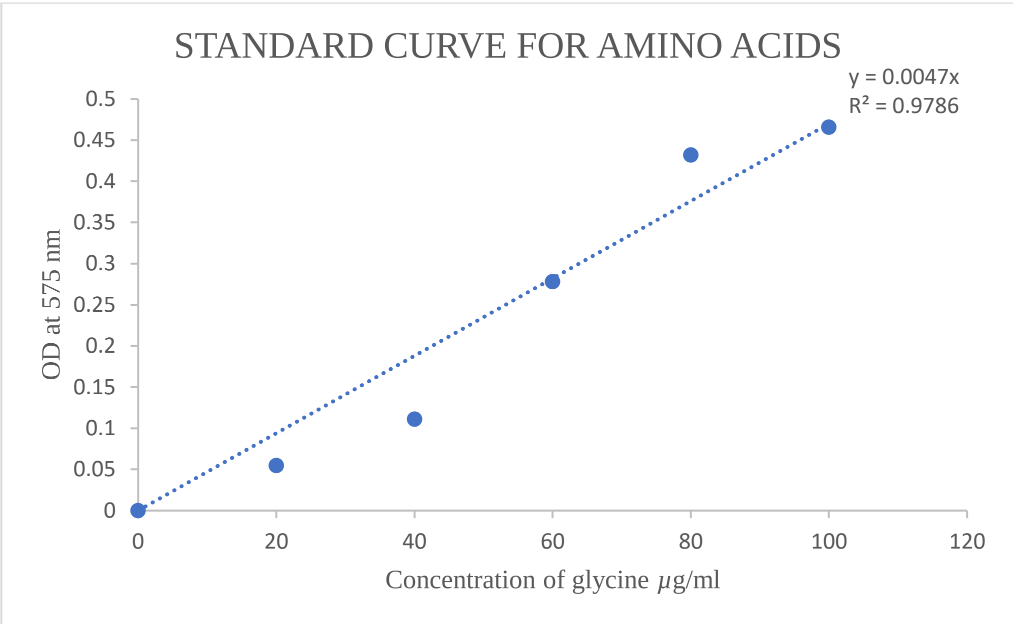

Figure 4.1.2 (C): Bar graph observed for carbohydrate concentration for samples treated with olive oil and kokum juice during the storage period.

### 4.1.3 Estimation of Amino acids:

Following a 21-day storage period, there was a significant difference ($p<0.05$) in the amino acid concentrations between the samples treated with olive oil and the samples treated with kokum juice. The concentrations of each sample declined significantly.

Furthermore, it also showed clear significance in the amino acid content across the days studied since $p<0.05$.

Figure 4.1.3(B) shows that until the conclusion of the storage period, the concentration of amino acids was significantly higher in the samples treated with kokum juice than in the samples treated with olive oil.

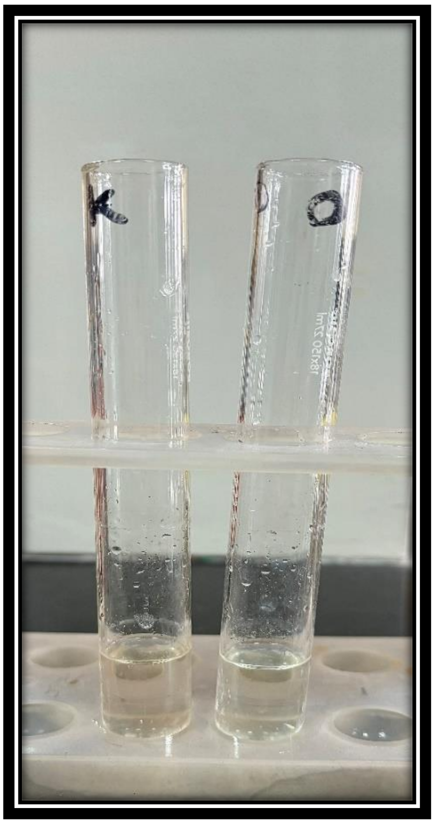



4.1.3 - STANDARD CURVE FOR AMINO ACIDS

DAY 0


DAY 7


DAY 14



DAY 21

FIGURE: 4.1.3(A) - ESTIMATION OF AMINO ACIDS

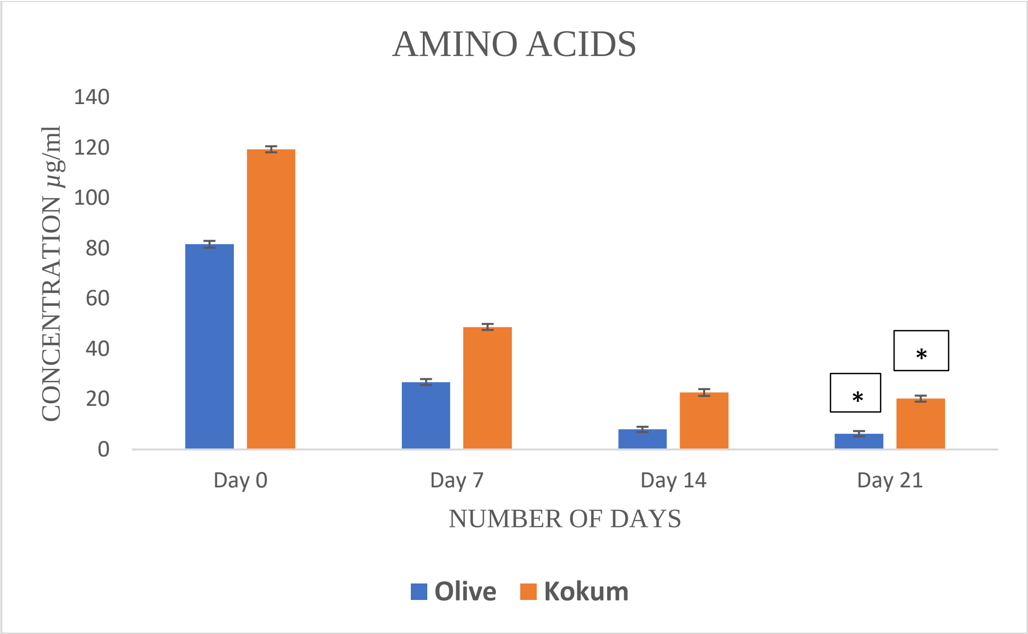



Figure 4.1.3 (B): Bar graph observed for the concentration of amino acids during the storage period.

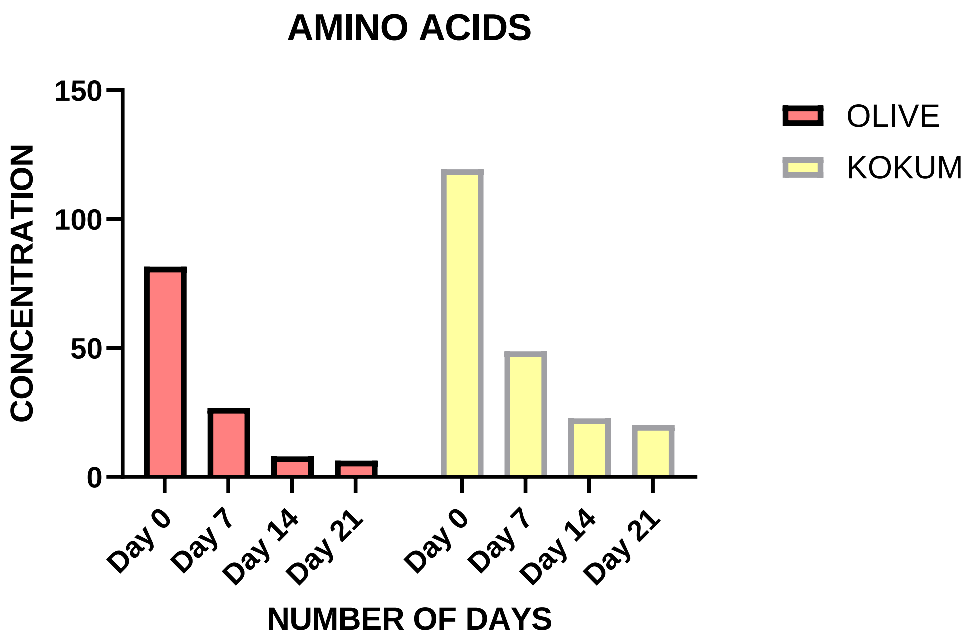
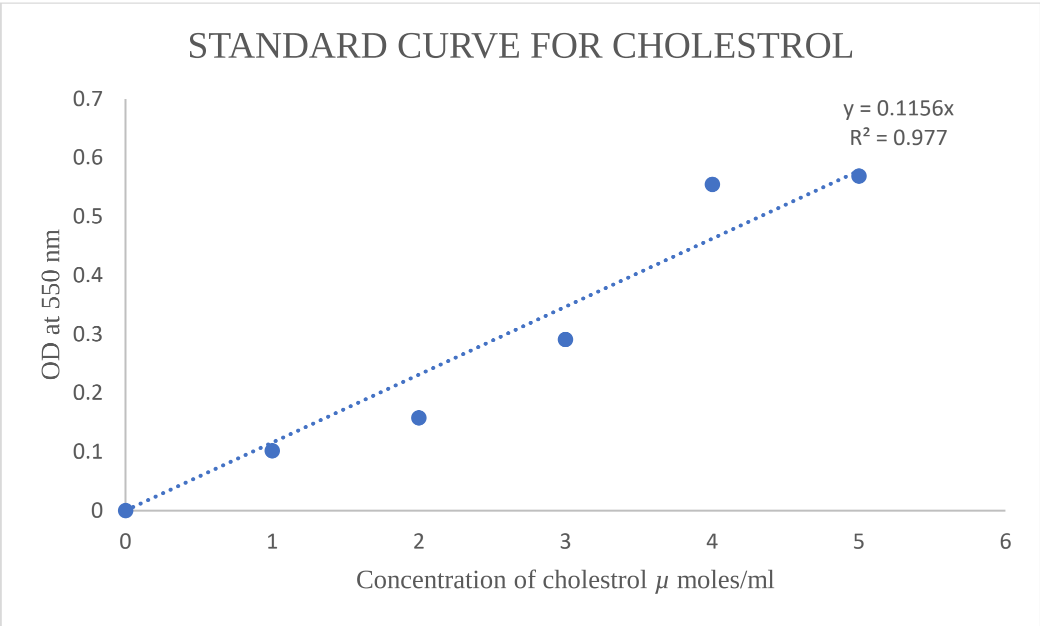



Figure 4.1.3 (C): Bar graph observed for amino acid concentration for samples treated with olive oil and kokum juice during the storage period.

4.1.4 Estimation of Cholesterol:

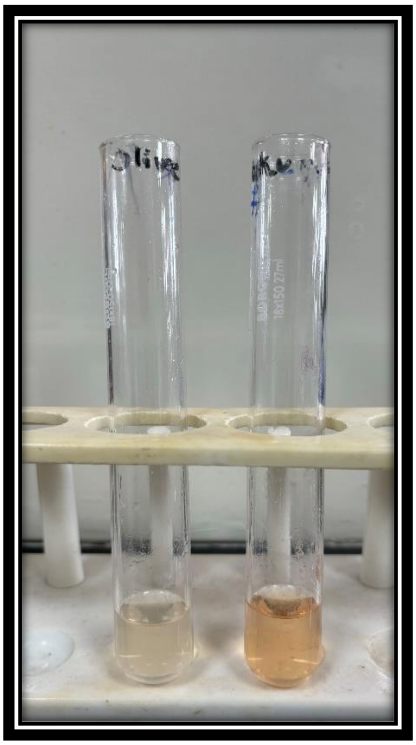
Since $p>0.05$, it does not show any significant variation between the control and experimental samples across the study period while compared day-wise as well as while compared between the 2 samples.

The cholesterol concentration in olive oil-treated samples grew during storage and was found to be quite high on the 21st day, but the cholesterol concentration reduced in samples treated with kokum juice, as shown in Figure 4.1.4(B). The cholesterol content showed variable levels in both the marinade samples studied.

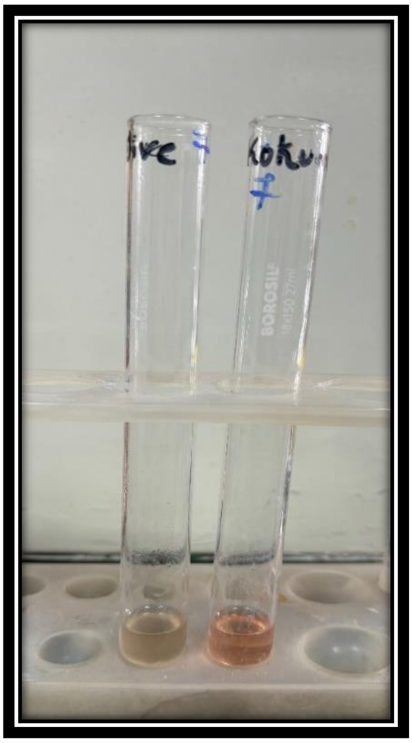



4.1.4 -STANDARD CURVE FOR CHOLESTEROL

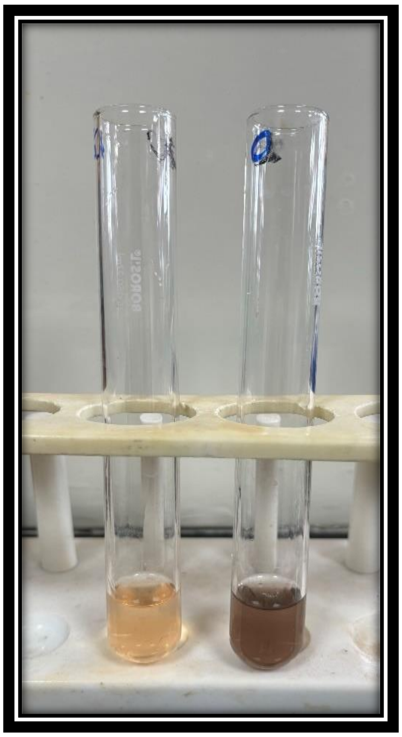
DAY 0



DAY 7



DAY 14



DAY 21

FIGURE: 4.1.4(A) - ESTIMATION OF CHOLESTROL

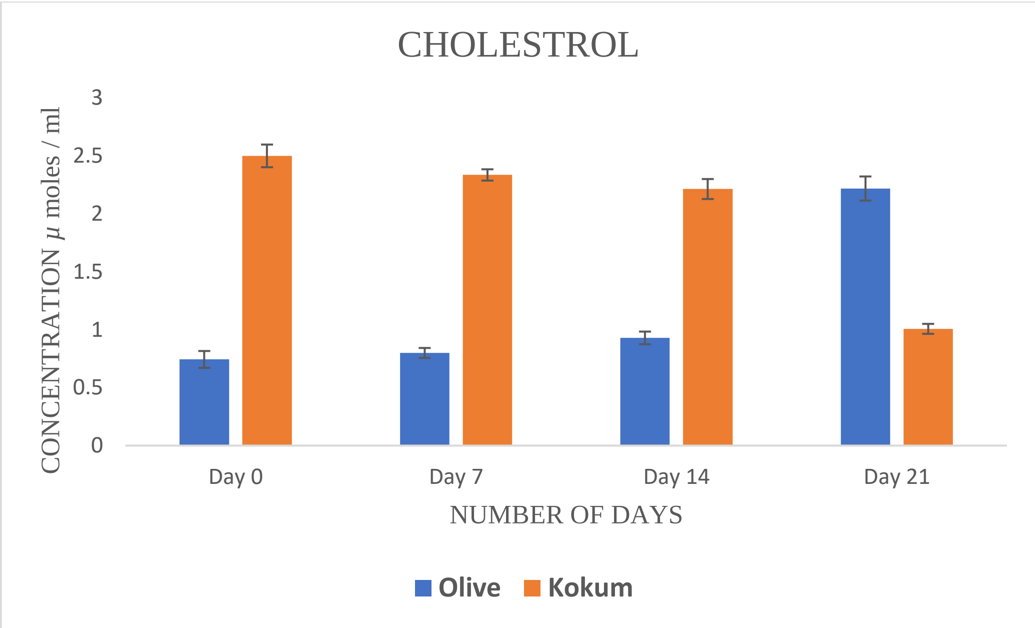



Figure 4.1.4 (B): Bar graph observed for the concentration of cholesterol during the storage period.

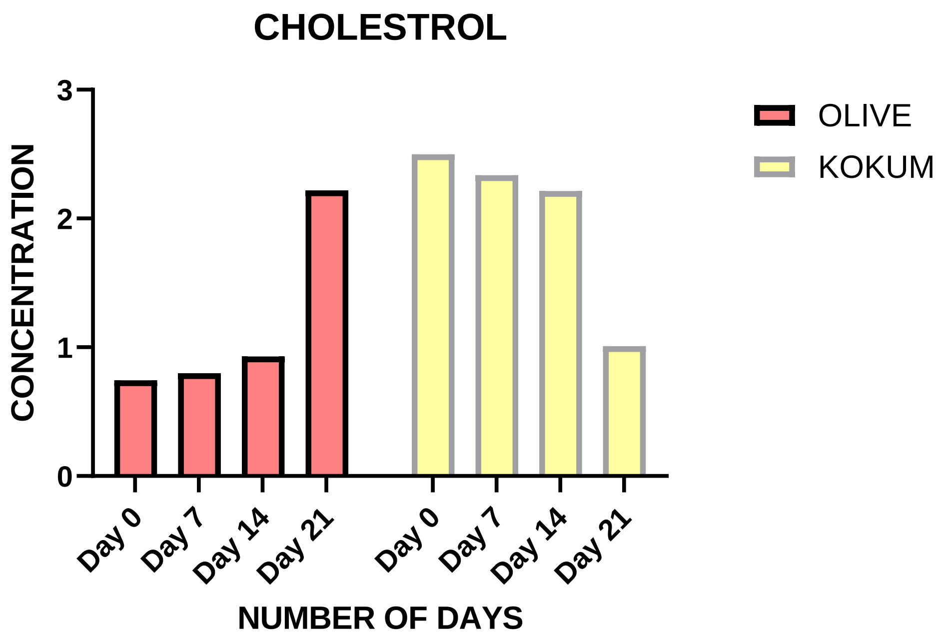



Figure 4.1.4(C): Bar graph observed for cholesterol concentration for samples treated with olive oil and kokum juice during the storage period.

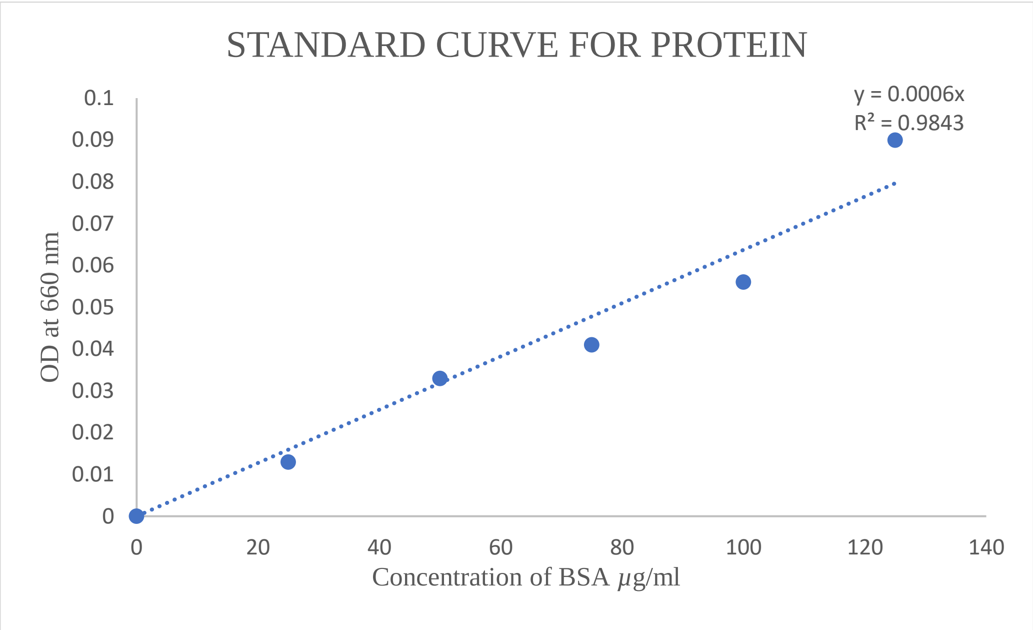
4.1.5 Estimation of Proteins:

Since p>0.05, there is no clear distinction among the protein content throughout the days studied.

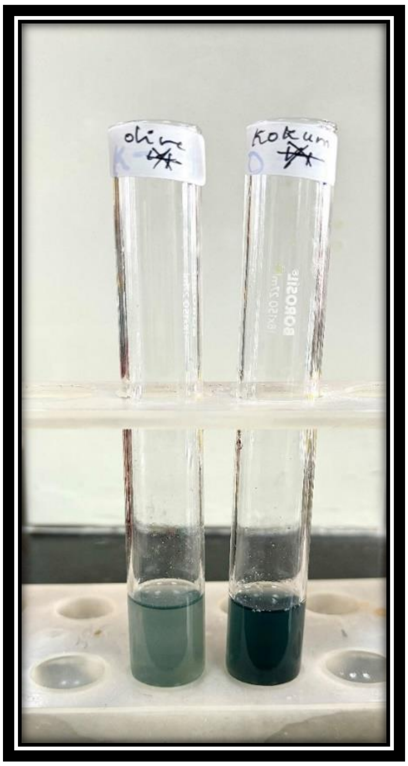
Since p>0.05, it does not show any significant variation across the marinade samples studied.

When comparing marinades with olive oil, it was shown that kokum juice had the highest protein concentration.

Over the course of the storage time shown in Figure 4.1.5 (B), a drop in the value of the protein content was noted for both of the samples treated with marinades.

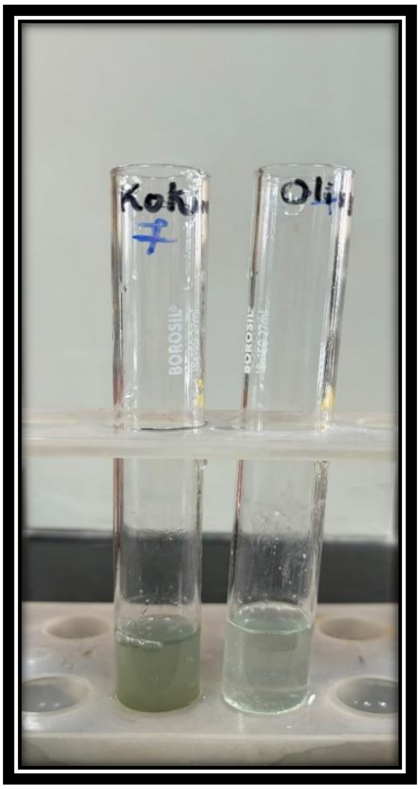



4.1.5 -STANDARD CURVE FOR PROTEINS

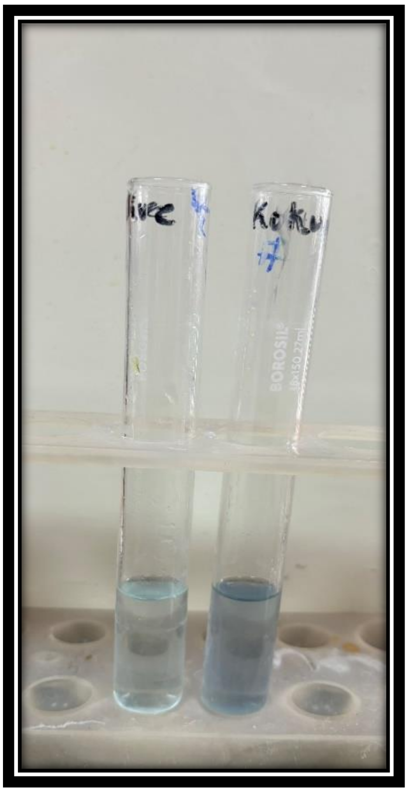


DAY 0

DAY 7



DAY 14



DAY 21

FIGURE: 4.1.5(A) - ESTIMATION OF PROTEIN

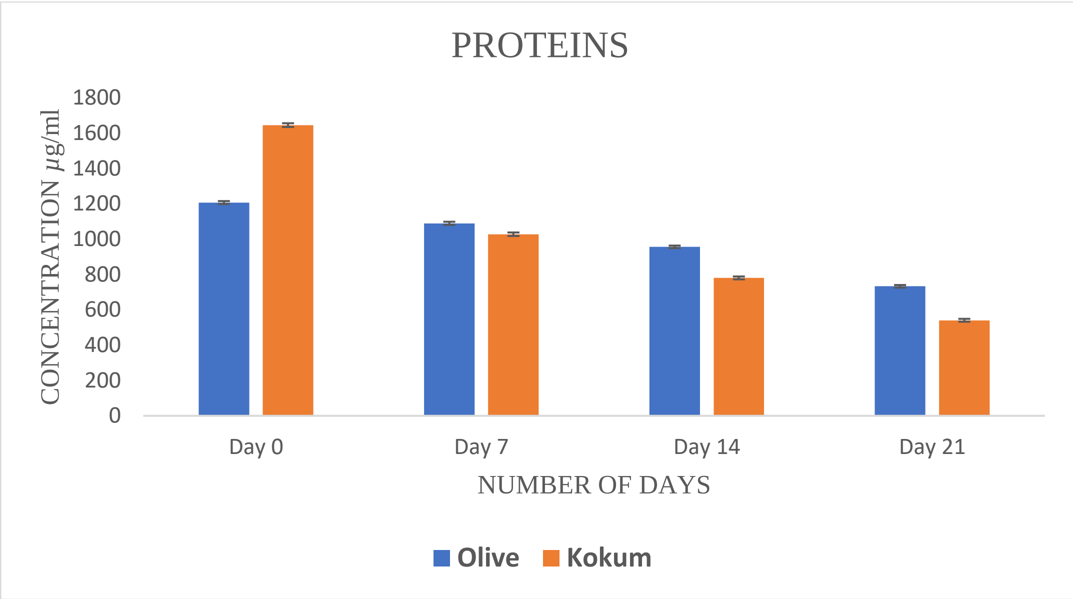



Figure 4.1.5 (B): Bar graph observed for the concentration of proteins during the storage period.

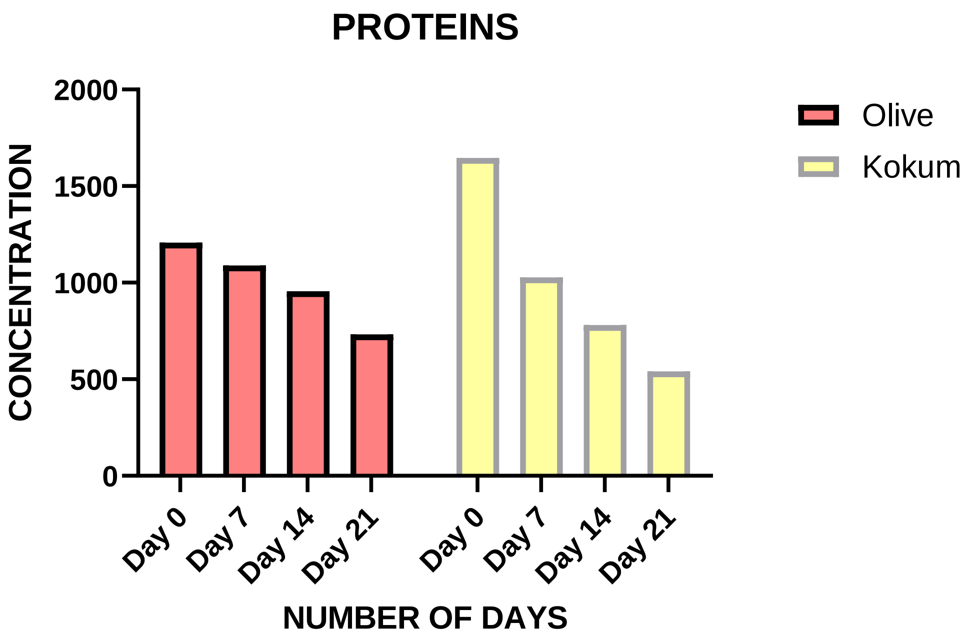


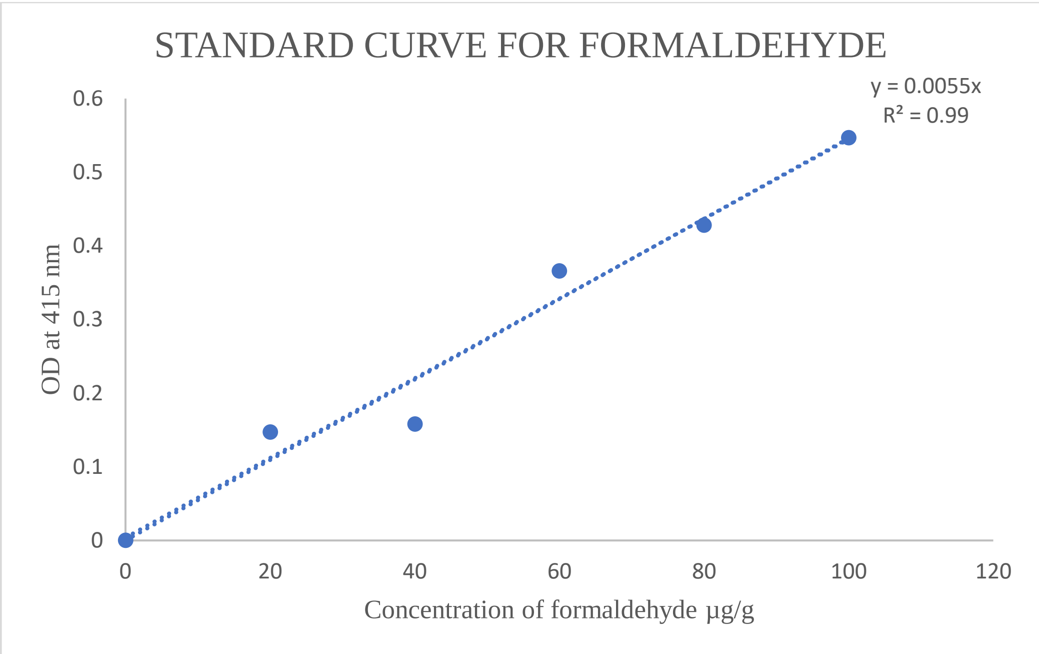

Figure 4.1.5 (C): Bar graph observed for protein concentration for samples treated with olive oil and kokum juice during the storage period.

### 4.1.6 Estimation of Formaldehyde:

Since $p<0.05$, clear variation was observed in the formaldehyde content across the days studied.

Since $p<0.05$, significant variation was seen in the formaldehyde content across the marinade samples studied.

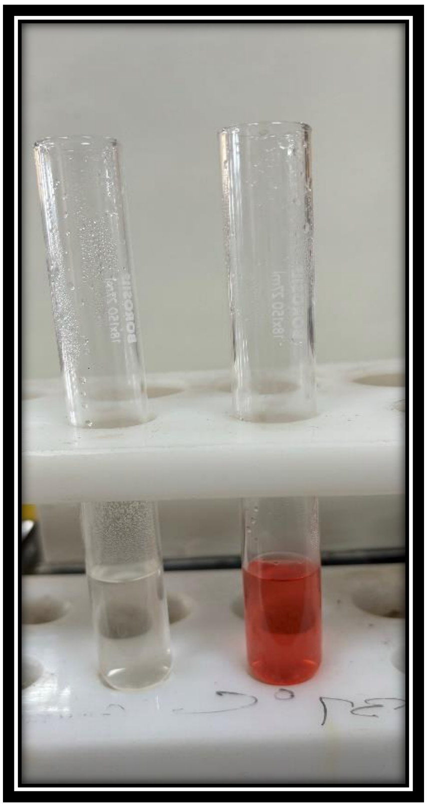
During the storage period, a rise in the formaldehyde levels was noted in both samples. After 21 days of storage, it was discovered that samples treated with olive oil had higher formaldehyde concentrations as shown in Figure 4.1.6 (B).




4.1.6- STANDARD CURVE FOR FORMALDEHYDE

DAY 0

DAY 7



DAY 14

DAY 21

FIGURE 4.1.6(A) - ESTIMATION OF FORMALDEHYDE

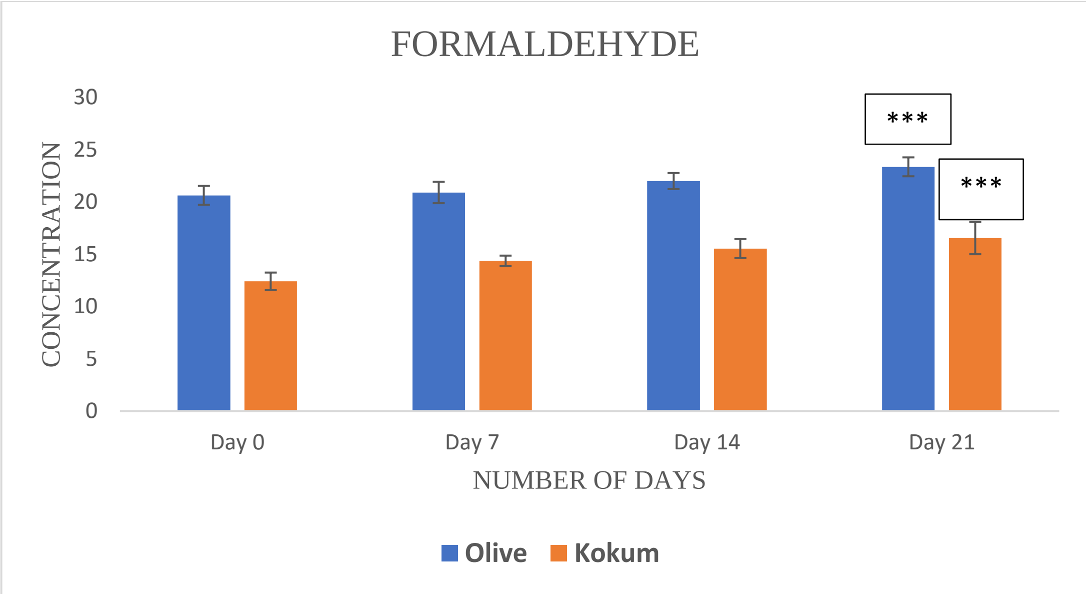



Figure 4.1.6 (B): Bar graph observed for the concentration of formaldehyde during the storage period.

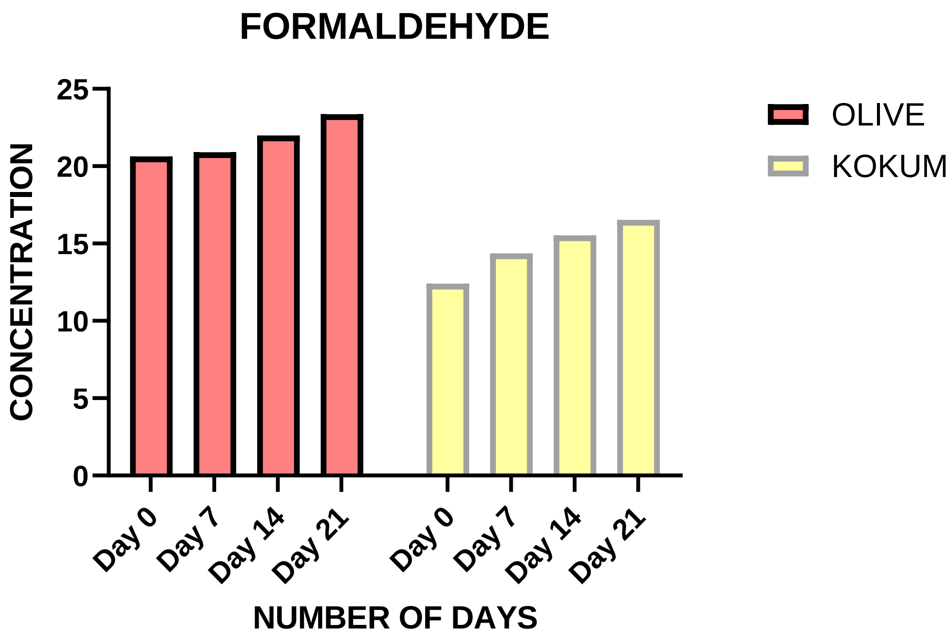



Figure 4.1.6 (C): Bar graph observed for formaldehyde concentration for samples treated with olive oil and kokum juice during the storage period.

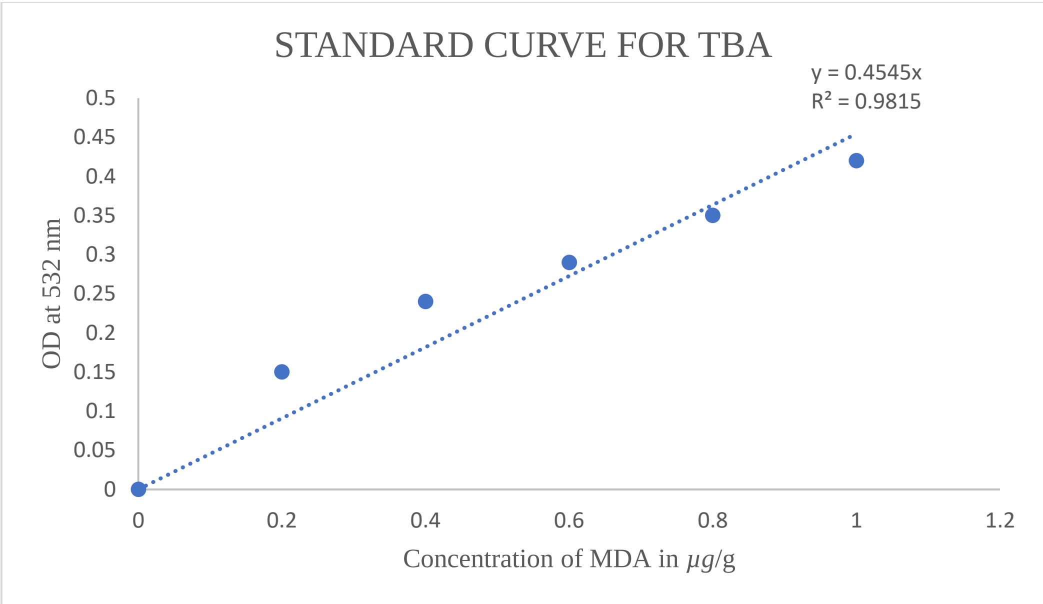
### 4.1.7 Estimation of Thiobarbituric acid:

Significant variation was observed in thiobarbituric acid content across the course of storage days since $p<0.05$.

Clear variance in the thiobarbituric acid content was noted across the marinade samples under study, with a significance level of $p<0.05$.

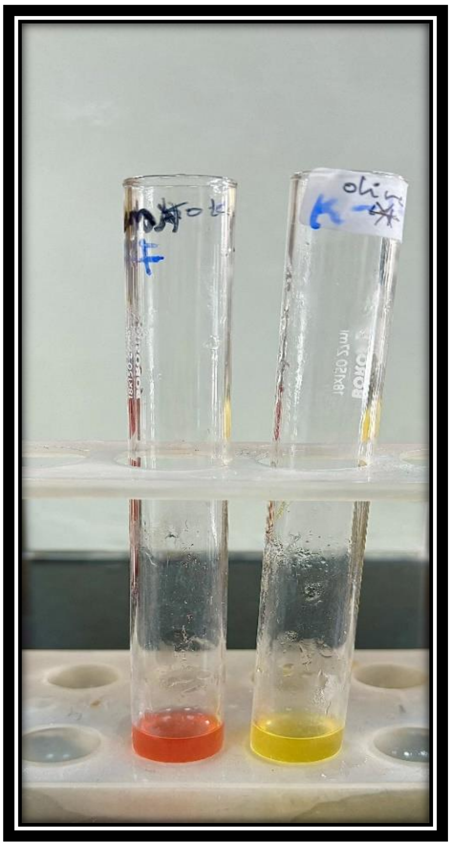
During the storage period, the amount of thiobarbituric acid content rose in both samples.

As shown in Figure 4.1.7 (B), samples treated with kokum juice had higher thiobarbituric acid values.

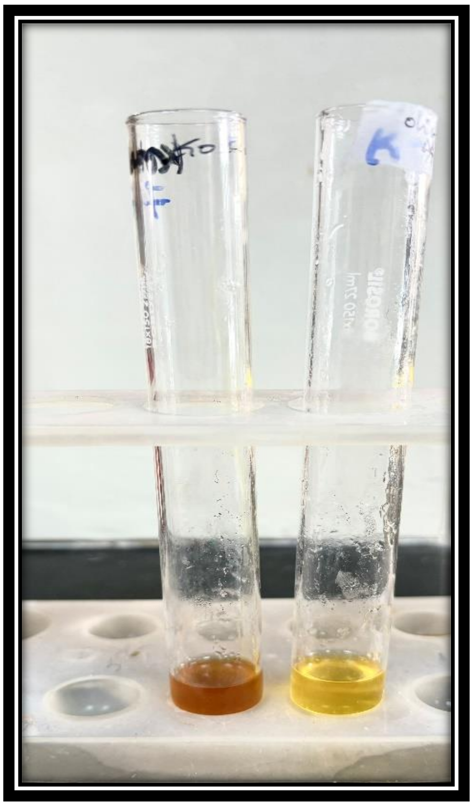



4.1.7-STANDARD CURVE FOR THIOBARBITURIC ACID

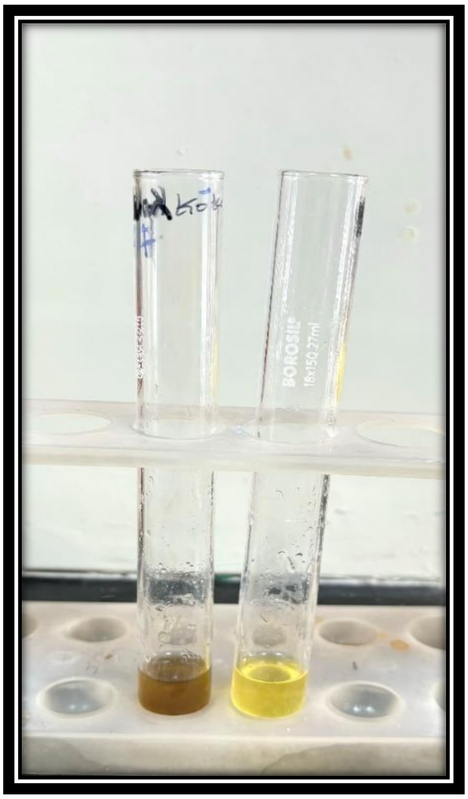
DAY 0



DAY 7



DAY 14



DAY 21

FIGURE 4.1.7(A) - ESTIMATION OF THIOBARBITURIC ACID

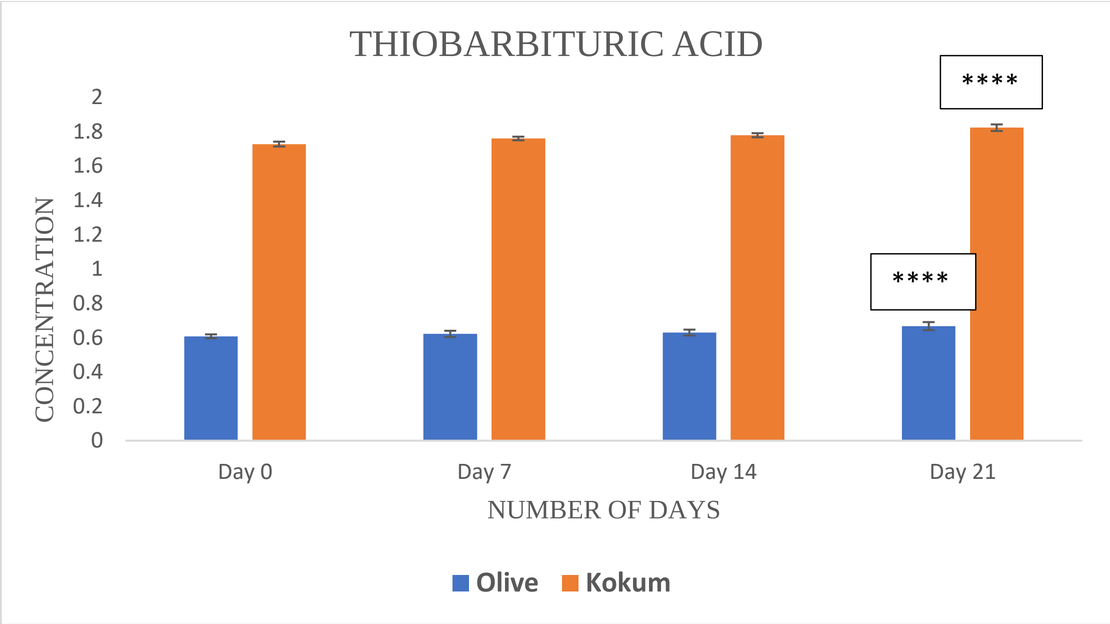



Figure 4.1.7 (B): Bar graph observed for the concentration of thiobarbituric acid during the storage period.

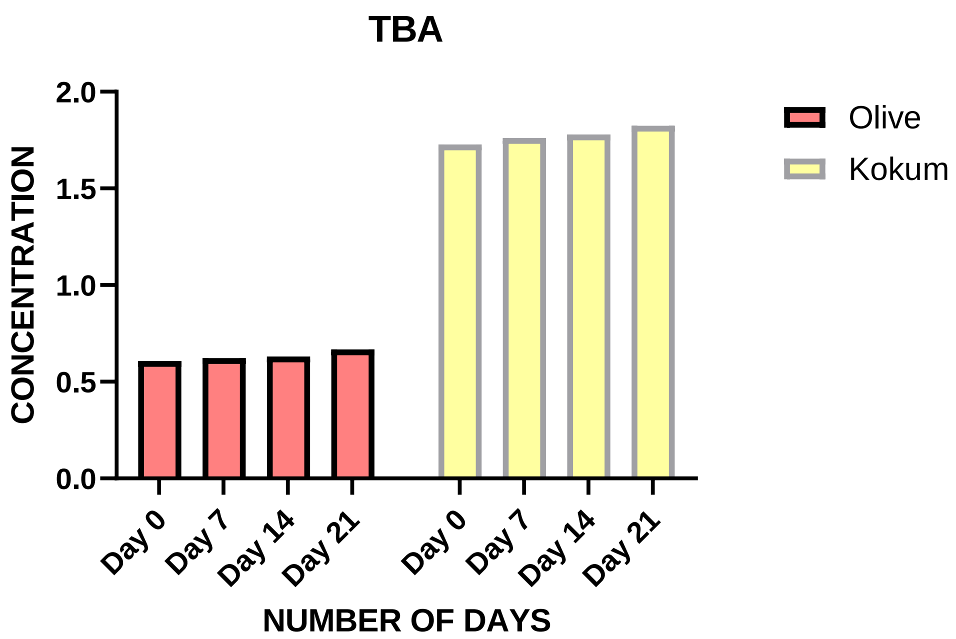



Figure 4.1.7 (C): Bar graph observed for thiobarbituric acid concentration for samples treated with olive oil and kokum juice during the storage period.

Sensory analysis of the mackerel fillets marinated with olive oil and kokum juice during the storage period of 21 days.

| | DAY 0 | | DAY 7 | | DAY 14 | | DAY 21 | |
|---|---|---|---|---|---|---|---|---|
| | OO | KJ | OO | KJ | OO | KJ | OO | KJ |
| COLOUR | 5.0 ± 0.0 | 5.0 ± 0.0 | 5.0 ± 0.0 | 5.0 ± 0.0 | 4.83 ± 0.41 | 5.0 ± 0.0 | 1.83 ± 0.41 | 5.0 ± 0.0 |
| ODOUR | 4.17 ± 0.75 | 4.83 ± 0.41 | 3.50 ± 0.55 | 4.67 ± 0.52 | 3.0 ± 0.89 | 4.33 ± 0.52 | 1.33 ± 0.52 | 3.67 ± 0.52 |
| TASTE | 3.0 ± 0.0 | 4.5 ± 0.55 | 2.83 ± 0.41 | 4.33 ± 0.52 | 2.33 ± 0.52 | 4.0 ± 0.0 | 1.17 ± 0.41 | 3.83 ± 0.41 |
| GENERAL APPEARANCE | 5.0 ± 0.0 | 5.0 ± 0.0 | 4.0 ± 0.63 | 5.0 ± 0.0 | 3.0 ± 0.63 | 5.0 ± 0.0 | 1.5 ± 0.55 | 4.67 ± 0.52 |
| TEXTURE | 4.67 ± 0.51 | 5.0 ± 0.0 | 3.33 ± 0.52 | 5.0 ± 0.0 | 2.5 ± 0.55 | 4.50 ± 0.55 | 1.17 ± 0.41 | 3.70 ± 0.47 |

Table 4.1.6: Changes in Sensory analysis for samples treated with olive oil and kokum juice during the storage period.

(OO- olive oil treated samples, KJ- kokum juice treated samples)

| | OLIVE OIL | KOKUM JUICE | P VALUE | * |
|---|---|---|---|---|
| COLOUR | 4.17 ± 1.56 | 5.0 ± 0.0 | 0.3625 | NS |
| ODOUR | 3.0 ± 1.21 | 4.38 ± 0.52 | 0.0298 | * |
| TASTE | 2.33 ± 0.83 | 4.17 ± 0.30 | 0.0072 | ** |
| GENERAL APPEARENCE | 3.4 ± 1.5 | 4.92 ± 0.17 | 0.1077 | NS |
| TEXTURE | 2.92 ± 1.48 | 4.55 ± 0.61 | 0.0410 | * |

Table 4.1.7: Data subjected to analysis of variance (ANOVA) to find the sensory effect of olive oil and kokum juice on marinated mackerel fillets.

| | P VALUE (ANOVA) | * | P VALUE (FRIEDMANS TEST) | * |
|---|---|---|---|---|
| COLOUR | 0.5000 | NS | >0.9999 | NS |
| ODOUR | 0.0877 | NS | 0.0417 | * |
| TASTE | 0.1430 | NS | 0.0417 | * |
| GENERAL APPEARANCE | 0.3843 | NS | 0.2500 | NS |
| TEXTURE | 0.1185 | NS | 0.0833 | NS |

Table 4.1.8 : Data subjected to analysis of variance (ANOVA) and Friedmans non-parametric test to find the day-wise sensory effect of olive oil and kokum juice on marinated mackerel fillets during the storage period (Day 0-Day 21).

| | PAIRED T TEST |
|---|---|
| COLOUR | p = 0.3625<br>NS |
| ODOUR | p = 0.0298<br>* |
| TASTE | p = 0.0072<br>** |
| GENERAL APPEARANCE | p = 0.1077<br>NS |
| TEXTURE | p = 0.0410<br>* |

Table 4.1.9: Data subjected to paired T test to compare the means of the sensory effect of olive oil and kokum juice on marinated mackerel fillets from day 0 to day 21.

| | ONE SAMPLE T TEST | |
|---|---|---|
| | OLIVE | KOKUM |
| COLOUR | p = 0.0128<br>* | --- |
| ODOUR | p= 0.0158<br>* | p = 0.0004<br>*** |
| TASTE | p =0.0110<br>* | p = 0.0001<br>*** |
| GENERAL APPEARANCE | p = 0.0202<br>* | p = <0.0001<br>**** |
| TEXTURE | p = 0.0282<br>* | p = 0.0007<br>*** |

Table 4.1.10 : Data subjected to One Sample T test to compare the means of the sensory effect of olive oil and kokum juice on marinated mackerel fillets.

4.1.8 <u>Assessment of Colour:</u>

Since $p<0.05$, clear variation was observed in the colour content across the days studied. Since $p<0.05$, significant variation was seen in the colour content scores across the marinade samples studied.

The colour content score ranked higher for samples treated with kokum juice than samples treated with olive oil.

As seen in Figure 4.1.8 (A), the color of the kokum juice held its quality until the conclusion of the storage time.

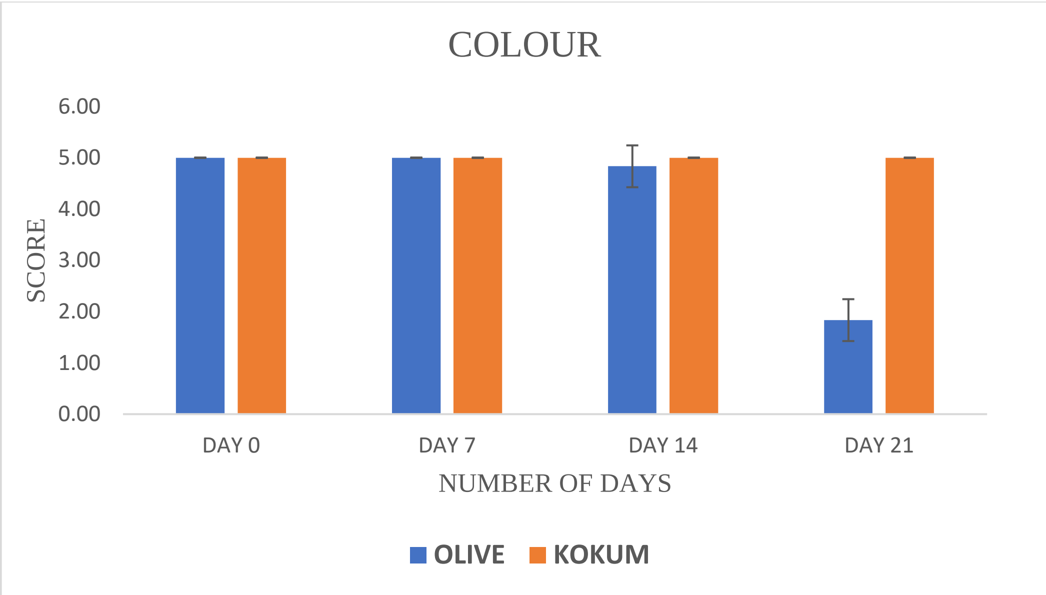



Figure 4.1.8 (A): Bar graph observed for the score of colour during the storage period.

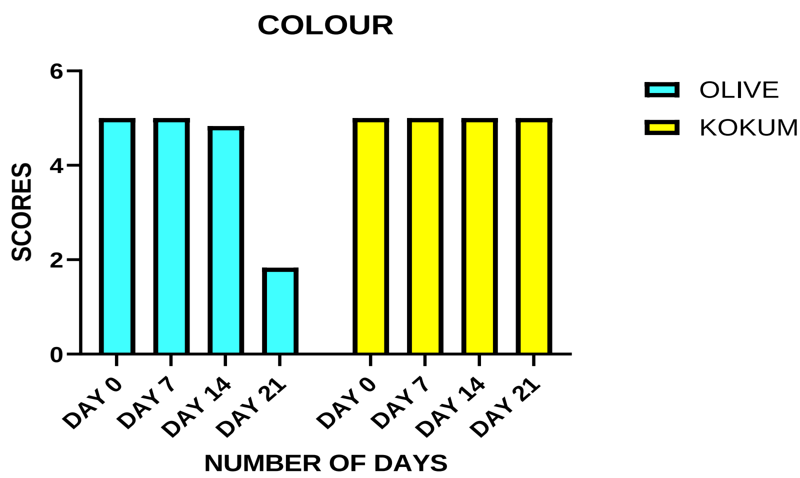



Figure 4.1.8 (B): Bar graph observed for colour scores for samples treated with olive oil and kokum juice during the storage period.

### 4.1.9 Assessment of Odour:

Since $p > 0.05$, there is no clear distinction among the odour scores across the days studied.

When the odour scores were compared between control sample (mackerel fillets treated with olive oil) and experimental sample (mackerel fillets treated with kokum juice), clear significant difference, $p < 0.05$ was detected at the end of storage period.

Figure 4.1.9 (A) shows that samples treated with kokum juice had a higher odor score than ones treated with olive oil.

The odour in both samples faded dramatically during storage.

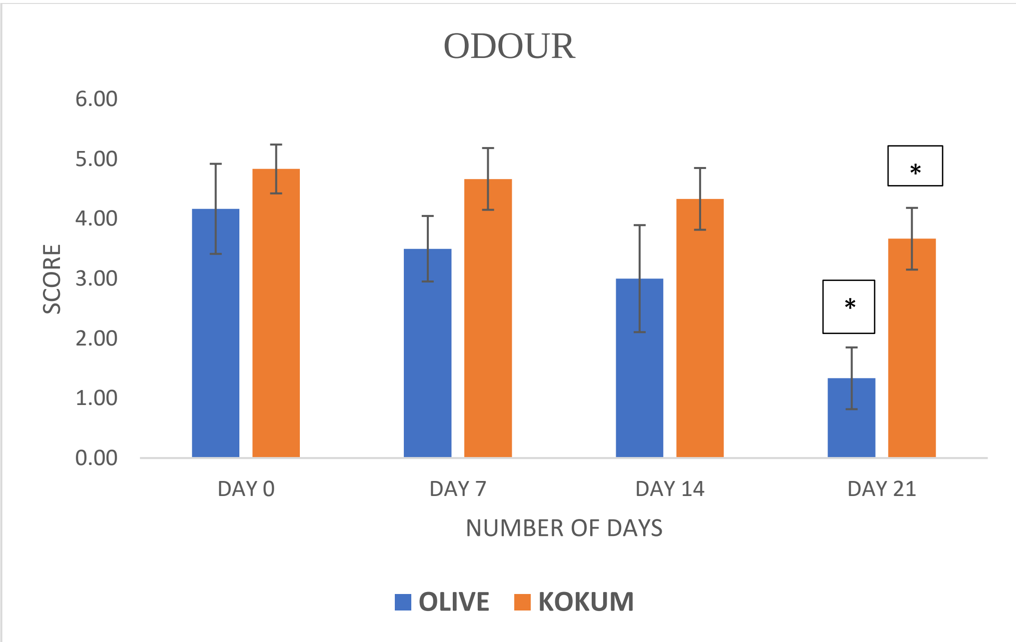



Figure 4.1.9(A): Bar graph observed for the score of odour during the storage period.

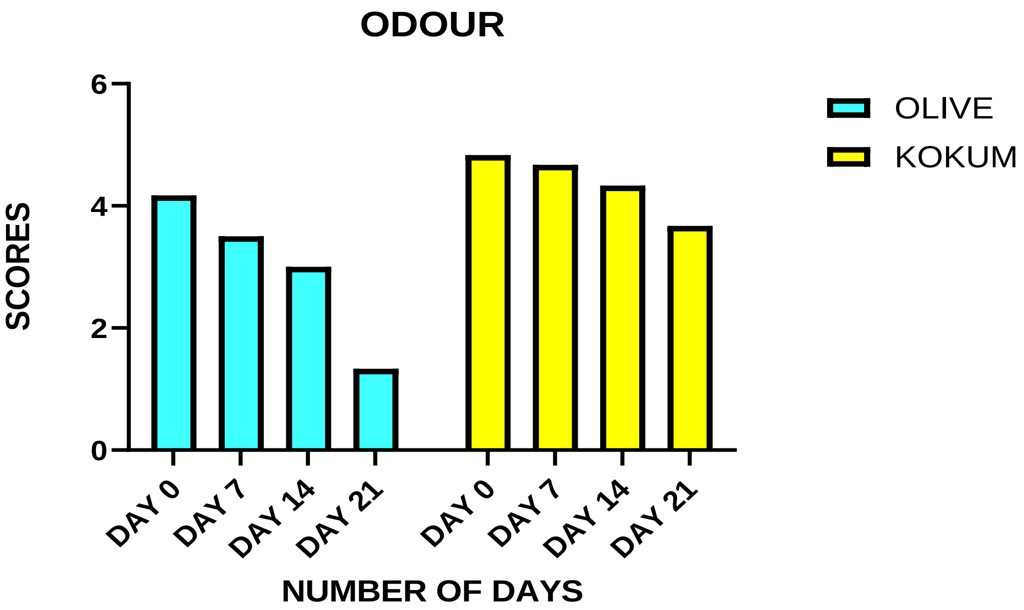



Figure 4.1.9(B): Bar graph observed for odour scores for samples treated with olive oil and kokum juice during the storage period.

4.1.10 Assessment of Flavour:

Clear significant variation was observed in taste across the days studied and between the two studied samples since $p<0.05$.

Up to the conclusion of the storage period, samples marinated with olive oil scored lower than those marinated with kokum juice, which was the highly preferred option. In the two marinades under investigation, there were differences in taste. As can be seen in Figure 4.1.10 (A), the taste quality declined over time in both samples.

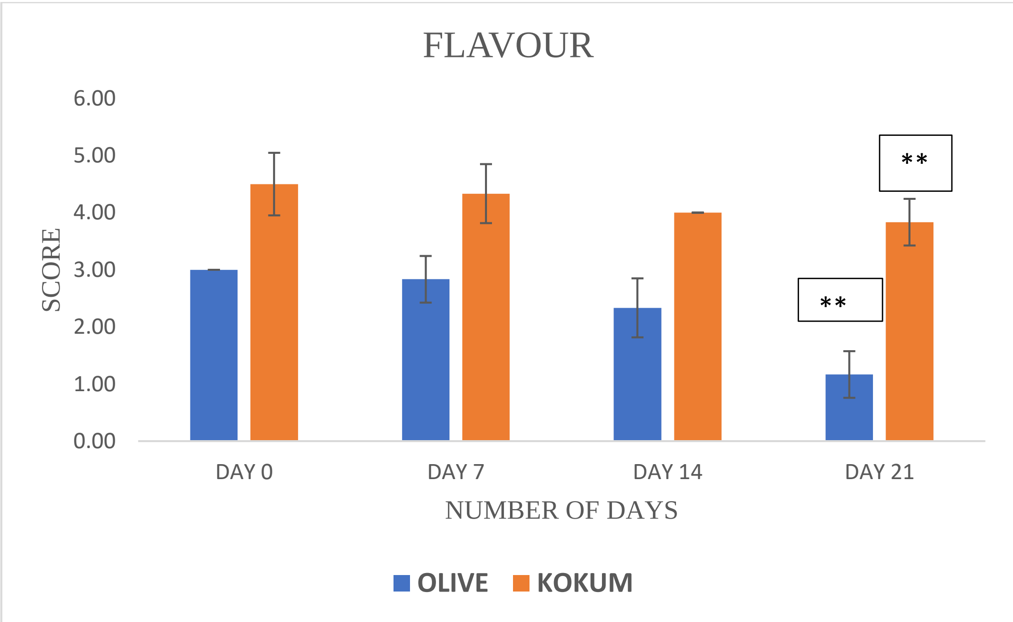



Figure 4.1.10 (A): Bar graph observed for the score of taste during the storage period.

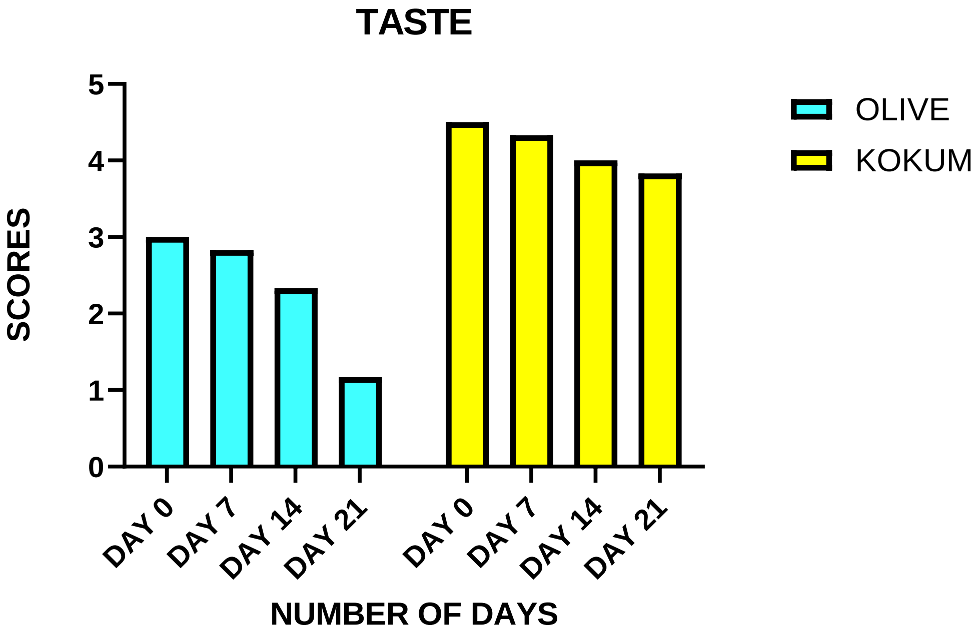



Figure 4.1.10(B): Bar graph observed for taste scores for samples treated with olive oil and kokum juice during the storage period.

4.1.11 Assessment of General appearance:

Since $p<0.05$, no clear variation was observed in the general appearance across the days studied.

Since $p<0.05$, no significant variation was seen in the scores of general appearances across the marinade samples studied.

Kokum juice retained its general appearance till the end of storage period while the fillets marinated in olive oil gradually lost their appearance.

As seen in Figure 4.1.11 (A), the samples marinated in kokum juice consistently scored higher.

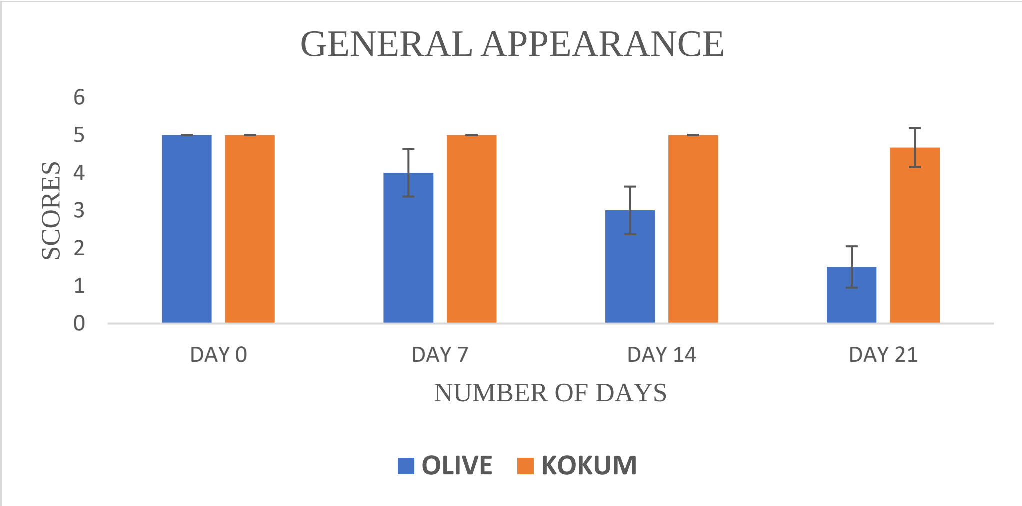



Figure 4.1.11(A): Bar graph observed for the score of general appearance during the storage period.

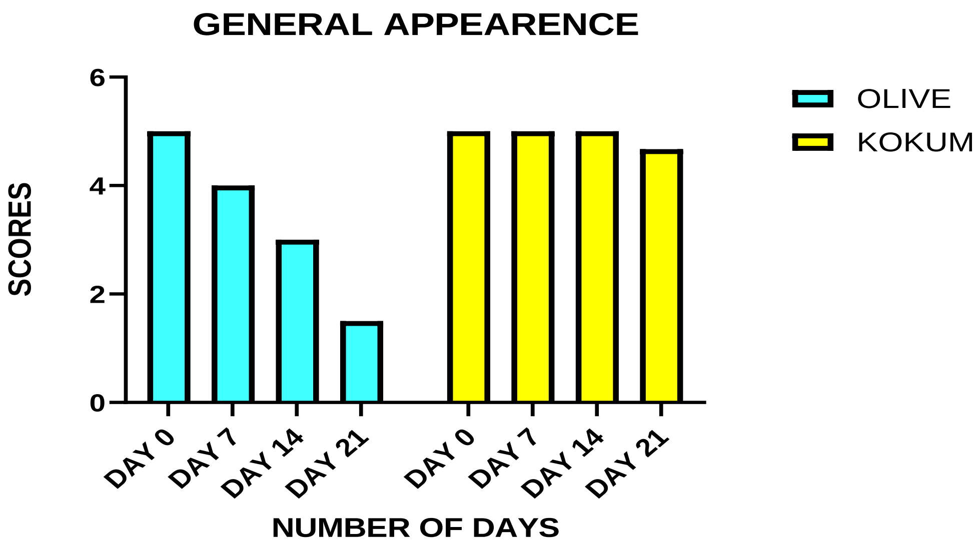



Figure 4.1.11(B): Bar graph observed for general appearance scores for samples treated with olive oil and kokum juice during the storage period.

4.1.12 Assessment of Texture:

At the conclusion of the storage period, a significant difference ($p<0.05$) in texture was found between the experimental sample (mackerel fillets treated with kokum juice) and the control sample (mackerel fillets treated with olive oil).

When compared day-wise, since $p>0.05$, there was no clear significant difference in the texture across the days studied.

Those marinated in kokum juice maintained their consistency and texture quality of flesh until the end of the storage period, while those treated with olive oil saw a decline in texture quality at that point. (Figure 4.1.12(A))

The scores ranked higher for kokum juice marinated samples.

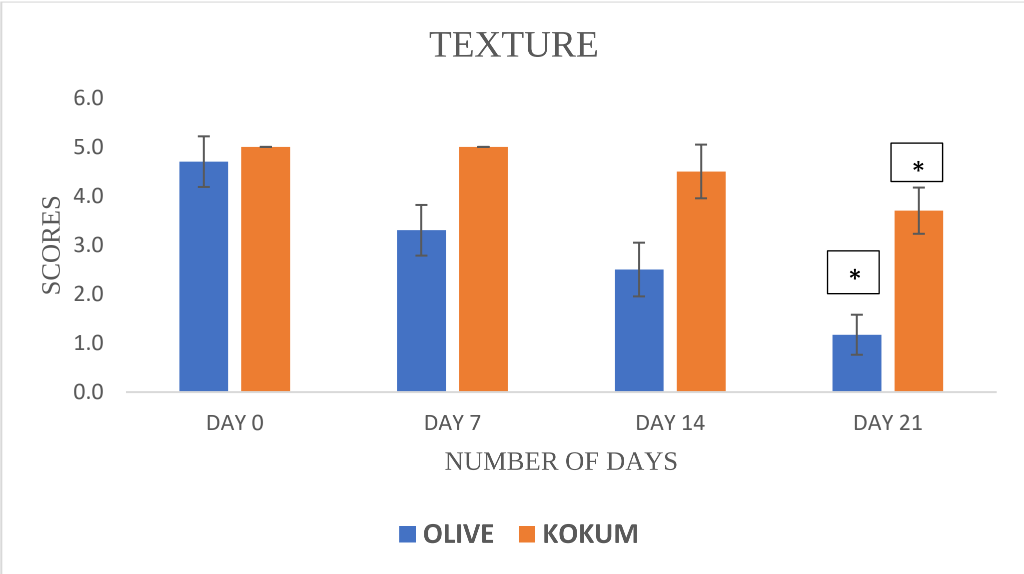



Figure 4.1.12(A): Bar graph observed for the score of texture during the storage period.

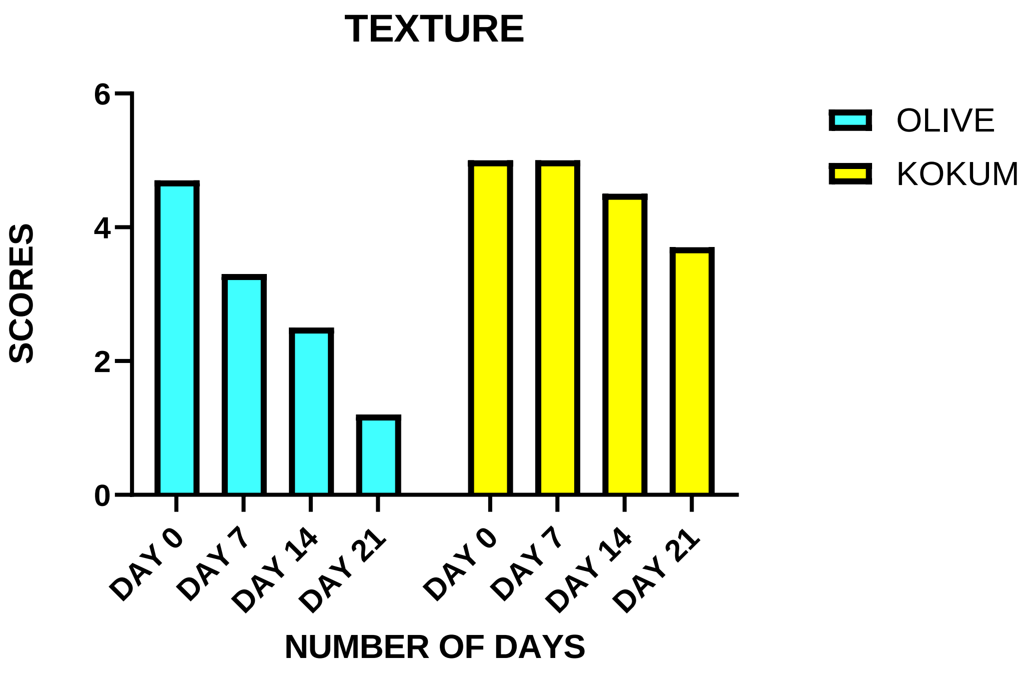



Figure 4.1.12(B): Bar graph observed for texture scores for samples treated with olive oil and kokum juice during the storage period.

DAY 0

DAY 7

DAY 14

DAY 21

FIGURE 4.1.13- SENSORY ANALYSIS OF MACKEREL FILLETS MARINATED WITH KOKUM

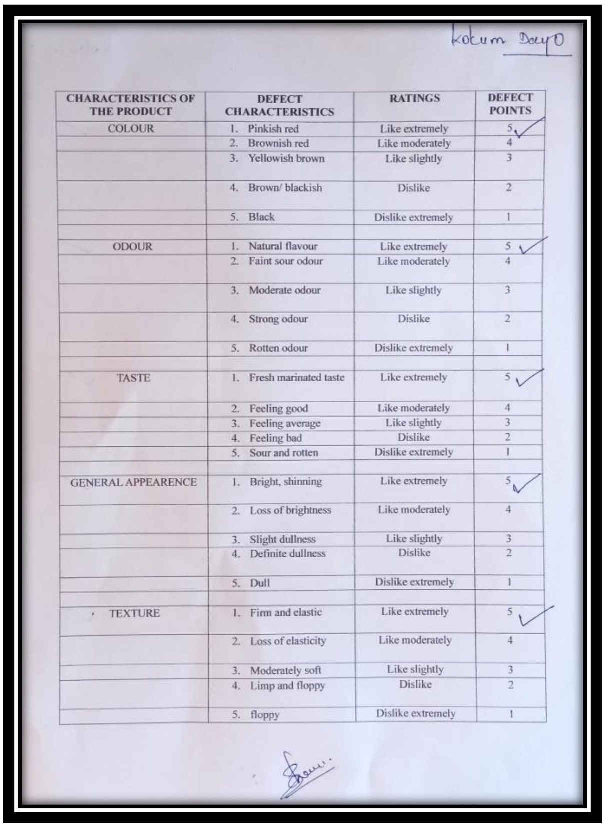
DAY 0

DAY 7

DAY 14

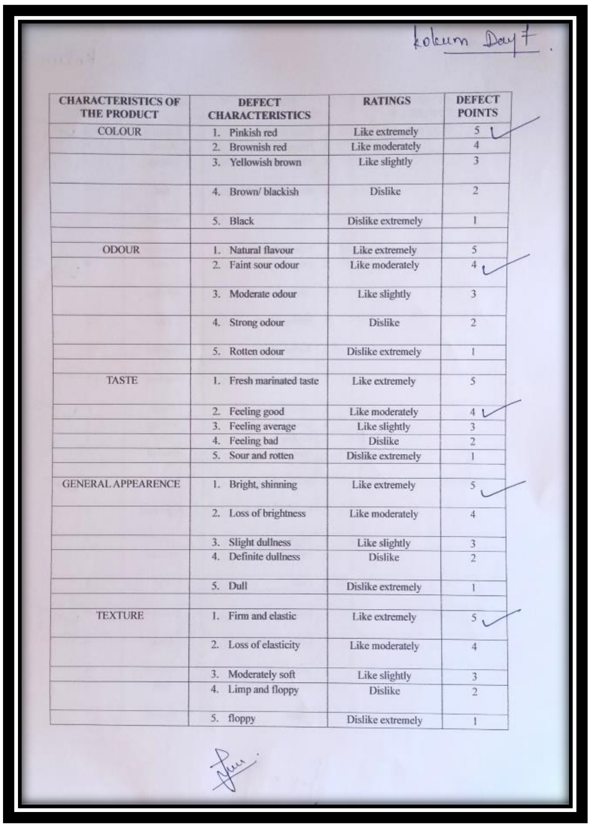
DAY 21

FIGURE 4.1.14- SENSORY ANALYSIS OF MACKEREL FILLETS MARINATED WITH OLIVE OIL



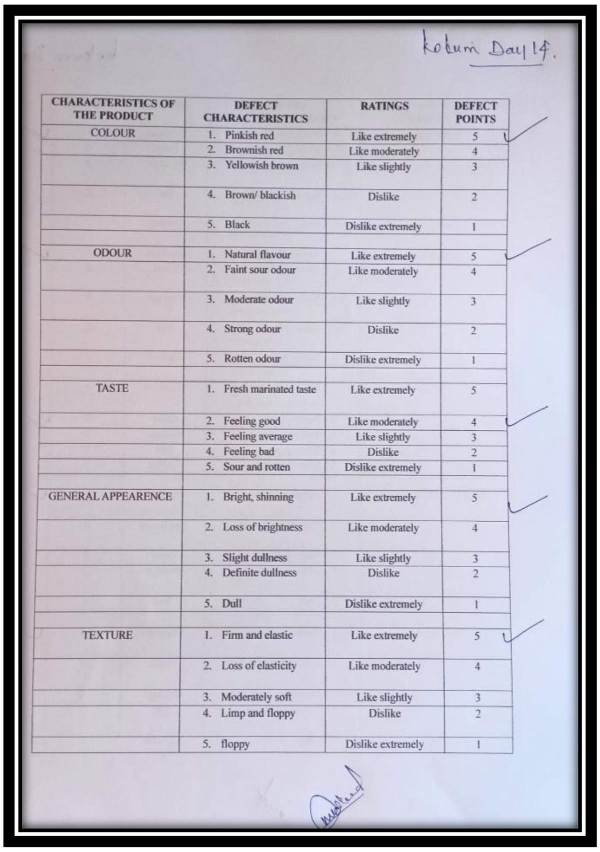
Kokum Day0

| CHARACTERISTICS OF THE PRODUCT | DEFECT CHARACTERISTICS | RATINGS | DEFECT POINTS |
|---|---|---|---|
| COLOUR | 1. Pinkish red | Like extremely | 5 ✓ |
| | 2. Brownish red | Like moderately | 4 |
| | 3. Yellowish brown | Like slightly | 3 |
| | 4. Brown/ blackish | Dislike | 2 |
| | 5. Black | Dislike extremely | 1 |
| ODOUR | 1. Natural flavour | Like extremely | 5 ✓ |
| | 2. Faint sour odour | Like moderately | 4 |
| | 3. Moderate odour | Like slightly | 3 |
| | 4. Strong odour | Dislike | 2 |
| | 5. Rotten odour | Dislike extremely | 1 |
| TASTE | 1. Fresh marinated taste | Like extremely | 5 ✓ |
| | 2. Feeling good | Like moderately | 4 |
| | 3. Feeling average | Like slightly | 3 |
| | 4. Feeling bad | Dislike | 2 |
| | 5. Sour and rotten | Dislike extremely | 1 |
| GENERAL APPEARENCE | 1. Bright, shinning | Like extremely | 5 ✓ |
| | 2. Loss of brightness | Like moderately | 4 |
| | 3. Slight dullness | Like slightly | 3 |
| | 4. Definite dullness | Dislike | 2 |
| | 5. Dull | Dislike extremely | 1 |
| TEXTURE | 1. Firm and elastic | Like extremely | 5 ✓ |
| | 2. Loss of elasticity | Like moderately | 4 |
| | 3. Moderately soft | Like slightly | 3 |
| | 4. Limp and floppy | Dislike | 2 |
| | 5. floppy | Dislike extremely | 1 |



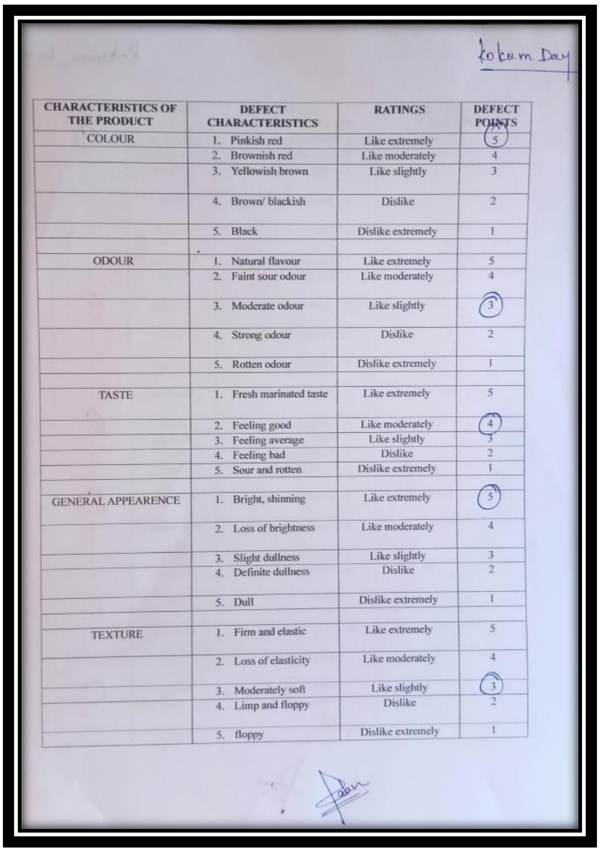
Kokum Day 7.

| CHARACTERISTICS OF THE PRODUCT | DEFECT CHARACTERISTICS | RATINGS | DEFECT POINTS |
|---|---|---|---|
| COLOUR | 1. Pinkish red | Like extremely | 5 ✓ |
| | 2. Brownish red | Like moderately | 4 |
| | 3. Yellowish brown | Like slightly | 3 |
| | 4. Brown/ blackish | Dislike | 2 |
| | 5. Black | Dislike extremely | 1 |
| ODOUR | 1. Natural flavour | Like extremely | 5 |
| | 2. Faint sour odour | Like moderately | 4 ✓ |
| | 3. Moderate odour | Like slightly | 3 |
| | 4. Strong odour | Dislike | 2 |
| | 5. Rotten odour | Dislike extremely | 1 |
| TASTE | 1. Fresh marinated taste | Like extremely | 5 |
| | 2. Feeling good | Like moderately | 4 ✓ |
| | 3. Feeling average | Like slightly | 3 |
| | 4. Feeling bad | Dislike | 2 |
| | 5. Sour and rotten | Dislike extremely | 1 |
| GENERAL APPEARENCE | 1. Bright, shinning | Like extremely | 5 ✓ |
| | 2. Loss of brightness | Like moderately | 4 |
| | 3. Slight dullness | Like slightly | 3 |
| | 4. Definite dullness | Dislike | 2 |
| | 5. Dull | Dislike extremely | 1 |
| TEXTURE | 1. Firm and elastic | Like extremely | 5 ✓ |
| | 2. Loss of elasticity | Like moderately | 4 |
| | 3. Moderately soft | Like slightly | 3 |
| | 4. Limp and floppy | Dislike | 2 |
| | 5. floppy | Dislike extremely | 1 |



Kokum Day 14.

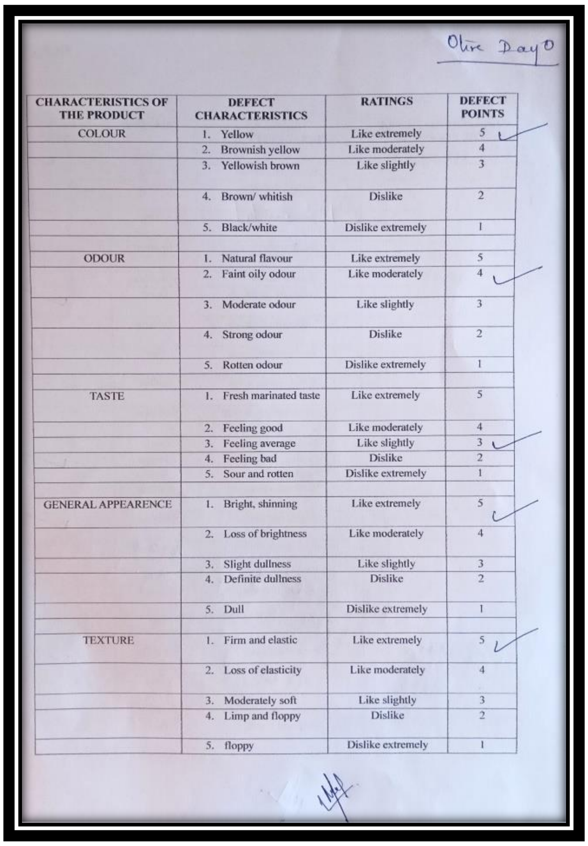
| CHARACTERISTICS OF THE PRODUCT | DEFECT CHARACTERISTICS | RATINGS | DEFECT POINTS |
|---|---|---|---|
| COLOUR | 1. Pinkish red | Like extremely | 5 ✓ |
| | 2. Brownish red | Like moderately | 4 |
| | 3. Yellowish brown | Like slightly | 3 |
| | 4. Brown/ blackish | Dislike | 2 |
| | 5. Black | Dislike extremely | 1 |
| ODOUR | 1. Natural flavour | Like extremely | 5 ✓ |
| | 2. Faint sour odour | Like moderately | 4 |
| | 3. Moderate odour | Like slightly | 3 |
| | 4. Strong odour | Dislike | 2 |
| | 5. Rotten odour | Dislike extremely | 1 |
| TASTE | 1. Fresh marinated taste | Like extremely | 5 |
| | 2. Feeling good | Like moderately | 4 ✓ |
| | 3. Feeling average | Like slightly | 3 |
| | 4. Feeling bad | Dislike | 2 |
| | 5. Sour and rotten | Dislike extremely | 1 |
| GENERAL APPEARENCE | 1. Bright, shinning | Like extremely | 5 ✓ |
| | 2. Loss of brightness | Like moderately | 4 |
| | 3. Slight dullness | Like slightly | 3 |
| | 4. Definite dullness | Dislike | 2 |
| | 5. Dull | Dislike extremely | 1 |
| TEXTURE | 1. Firm and elastic | Like extremely | 5 ✓ |
| | 2. Loss of elasticity | Like moderately | 4 |
| | 3. Moderately soft | Like slightly | 3 |
| | 4. Limp and floppy | Dislike | 2 |
| | 5. floppy | Dislike extremely | 1 |



Kokum Day

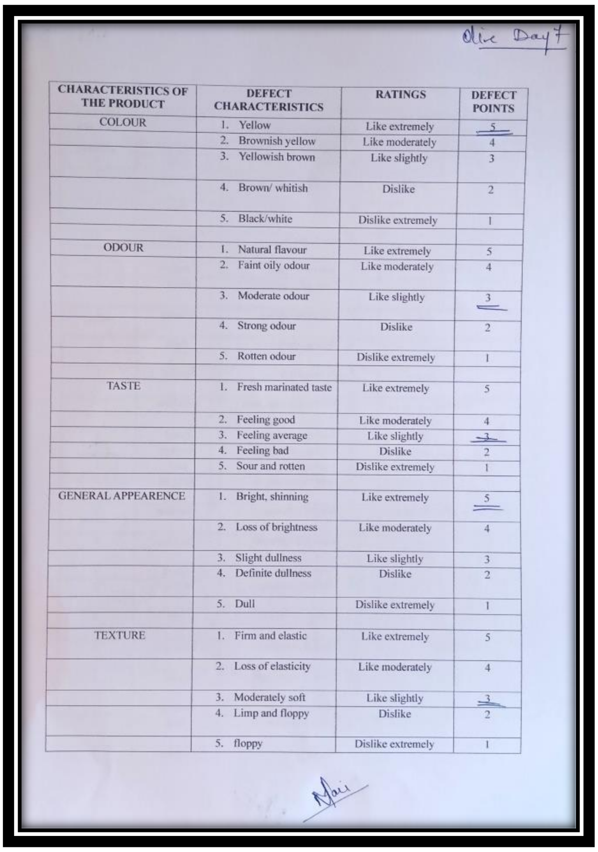
| CHARACTERISTICS OF THE PRODUCT | DEFECT CHARACTERISTICS | RATINGS | DEFECT POINTS |
|---|---|---|---|
| COLOUR | 1. Pinkish red | Like extremely | (5) |
| | 2. Brownish red | Like moderately | 4 |
| | 3. Yellowish brown | Like slightly | 3 |
| | 4. Brown/ blackish | Dislike | 2 |
| | 5. Black | Dislike extremely | 1 |
| ODOUR | 1. Natural flavour | Like extremely | 5 |
| | 2. Faint sour odour | Like moderately | 4 |
| | 3. Moderate odour | Like slightly | (3) |
| | 4. Strong odour | Dislike | 2 |
| | 5. Rotten odour | Dislike extremely | 1 |
| TASTE | 1. Fresh marinated taste | Like extremely | 5 |
| | 2. Feeling good | Like moderately | (4) |
| | 3. Feeling average | Like slightly | 3 |
| | 4. Feeling bad | Dislike | 2 |
| | 5. Sour and rotten | Dislike extremely | 1 |
| GENERAL APPEARENCE | 1. Bright, shinning | Like extremely | (5) |
| | 2. Loss of brightness | Like moderately | 4 |
| | 3. Slight dullness | Like slightly | 3 |
| | 4. Definite dullness | Dislike | 2 |
| | 5. Dull | Dislike extremely | 1 |
| TEXTURE | 1. Firm and elastic | Like extremely | 5 |
| | 2. Loss of elasticity | Like moderately | 4 |
| | 3. Moderately soft | Like slightly | (3) |
| | 4. Limp and floppy | Dislike | 2 |
| | 5. floppy | Dislike extremely | 1 |

FIGURE 4.1.15- SENSORY ANALYSIS OF MACKEREL FILLETS MARINATED WITH KOKUM JUICE USING 5-POINT HEDONIC SCALE.



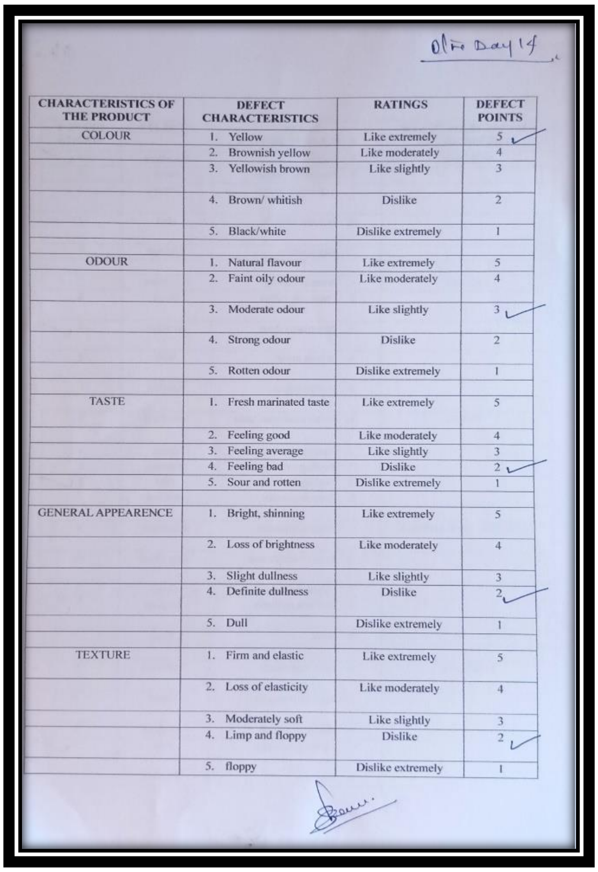
Olive Day 0

| CHARACTERISTICS OF THE PRODUCT | DEFECT CHARACTERISTICS | RATINGS | DEFECT POINTS |
|---|---|---|---|
| COLOUR | 1. Yellow | Like extremely | 5 |
| | 2. Brownish yellow | Like moderately | 4 |
| | 3. Yellowish brown | Like slightly | 3 |
| | 4. Brown/ whitish | Dislike | 2 |
| | 5. Black/white | Dislike extremely | 1 |
| ODOUR | 1. Natural flavour | Like extremely | 5 |
| | 2. Faint oily odour | Like moderately | 4 |
| | 3. Moderate odour | Like slightly | 3 |
| | 4. Strong odour | Dislike | 2 |
| | 5. Rotten odour | Dislike extremely | 1 |
| TASTE | 1. Fresh marinated taste | Like extremely | 5 |
| | 2. Feeling good | Like moderately | 4 |
| | 3. Feeling average | Like slightly | 3 |
| | 4. Feeling bad | Dislike | 2 |
| | 5. Sour and rotten | Dislike extremely | 1 |
| GENERAL APPEARENCE | 1. Bright, shinning | Like extremely | 5 |
| | 2. Loss of brightness | Like moderately | 4 |
| | 3. Slight dullness | Like slightly | 3 |
| | 4. Definite dullness | Dislike | 2 |
| | 5. Dull | Dislike extremely | 1 |
| TEXTURE | 1. Firm and elastic | Like extremely | 5 |
| | 2. Loss of elasticity | Like moderately | 4 |
| | 3. Moderately soft | Like slightly | 3 |
| | 4. Limp and floppy | Dislike | 2 |
| | 5. floppy | Dislike extremely | 1 |



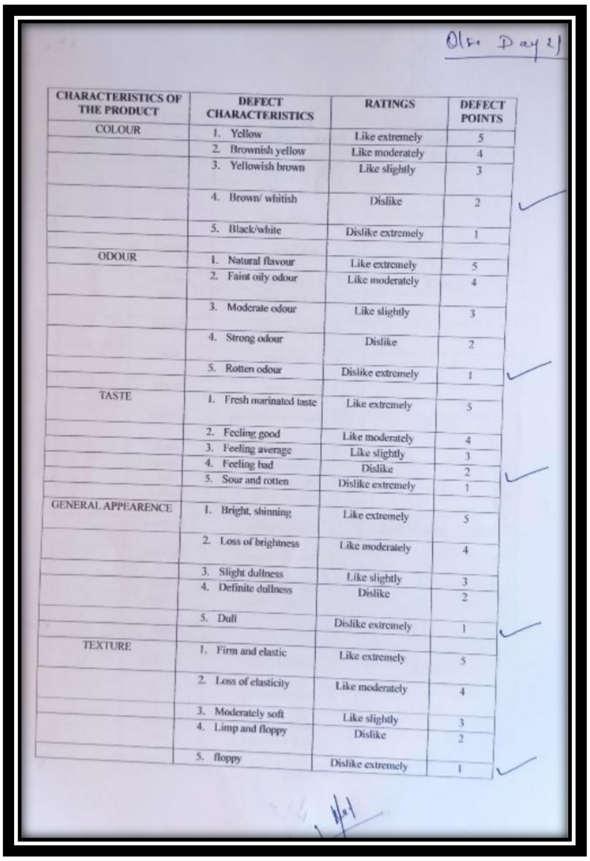
Olive Day 7

| CHARACTERISTICS OF THE PRODUCT | DEFECT CHARACTERISTICS | RATINGS | DEFECT POINTS |
|---|---|---|---|
| COLOUR | 1. Yellow | Like extremely | 5 |
| | 2. Brownish yellow | Like moderately | 4 |
| | 3. Yellowish brown | Like slightly | 3 |
| | 4. Brown/ whitish | Dislike | 2 |
| | 5. Black/white | Dislike extremely | 1 |
| ODOUR | 1. Natural flavour | Like extremely | 5 |
| | 2. Faint oily odour | Like moderately | 4 |
| | 3. Moderate odour | Like slightly | 3 |
| | 4. Strong odour | Dislike | 2 |
| | 5. Rotten odour | Dislike extremely | 1 |
| TASTE | 1. Fresh marinated taste | Like extremely | 5 |
| | 2. Feeling good | Like moderately | 4 |
| | 3. Feeling average | Like slightly | 3 |
| | 4. Feeling bad | Dislike | 2 |
| | 5. Sour and rotten | Dislike extremely | 1 |
| GENERAL APPEARENCE | 1. Bright, shinning | Like extremely | 5 |
| | 2. Loss of brightness | Like moderately | 4 |
| | 3. Slight dullness | Like slightly | 3 |
| | 4. Definite dullness | Dislike | 2 |
| | 5. Dull | Dislike extremely | 1 |
| TEXTURE | 1. Firm and elastic | Like extremely | 5 |
| | 2. Loss of elasticity | Like moderately | 4 |
| | 3. Moderately soft | Like slightly | 3 |
| | 4. Limp and floppy | Dislike | 2 |
| | 5. floppy | Dislike extremely | 1 |



Olive Day 14

| CHARACTERISTICS OF THE PRODUCT | DEFECT CHARACTERISTICS | RATINGS | DEFECT POINTS |
|---|---|---|---|
| COLOUR | 1. Yellow | Like extremely | 5 |
| | 2. Brownish yellow | Like moderately | 4 |
| | 3. Yellowish brown | Like slightly | 3 |
| | 4. Brown/ whitish | Dislike | 2 |
| | 5. Black/white | Dislike extremely | 1 |
| ODOUR | 1. Natural flavour | Like extremely | 5 |
| | 2. Faint oily odour | Like moderately | 4 |
| | 3. Moderate odour | Like slightly | 3 |
| | 4. Strong odour | Dislike | 2 |
| | 5. Rotten odour | Dislike extremely | 1 |
| TASTE | 1. Fresh marinated taste | Like extremely | 5 |
| | 2. Feeling good | Like moderately | 4 |
| | 3. Feeling average | Like slightly | 3 |
| | 4. Feeling bad | Dislike | 2 |
| | 5. Sour and rotten | Dislike extremely | 1 |
| GENERAL APPEARENCE | 1. Bright, shinning | Like extremely | 5 |
| | 2. Loss of brightness | Like moderately | 4 |
| | 3. Slight dullness | Like slightly | 3 |
| | 4. Definite dullness | Dislike | 2 |
| | 5. Dull | Dislike extremely | 1 |
| TEXTURE | 1. Firm and elastic | Like extremely | 5 |
| | 2. Loss of elasticity | Like moderately | 4 |
| | 3. Moderately soft | Like slightly | 3 |
| | 4. Limp and floppy | Dislike | 2 |
| | 5. floppy | Dislike extremely | 1 |



Olive Day 21

| CHARACTERISTICS OF THE PRODUCT | DEFECT CHARACTERISTICS | RATINGS | DEFECT POINTS |
|---|---|---|---|
| COLOUR | 1. Yellow | Like extremely | 5 |
| | 2. Brownish yellow | Like moderately | 4 |
| | 3. Yellowish brown | Like slightly | 3 |
| | 4. Brown/ whitish | Dislike | 2 |
| | 5. Black/white | Dislike extremely | 1 |
| ODOUR | 1. Natural flavour | Like extremely | 5 |
| | 2. Faint oily odour | Like moderately | 4 |
| | 3. Moderate odour | Like slightly | 3 |
| | 4. Strong odour | Dislike | 2 |
| | 5. Rotten odour | Dislike extremely | 1 |
| TASTE | 1. Fresh marinated taste | Like extremely | 5 |
| | 2. Feeling good | Like moderately | 4 |
| | 3. Feeling average | Like slightly | 3 |
| | 4. Feeling bad | Dislike | 2 |
| | 5. Sour and rotten | Dislike extremely | 1 |
| GENERAL APPEARENCE | 1. Bright, shinning | Like extremely | 5 |
| | 2. Loss of brightness | Like moderately | 4 |
| | 3. Slight dullness | Like slightly | 3 |
| | 4. Definite dullness | Dislike | 2 |
| | 5. Dull | Dislike extremely | 1 |
| TEXTURE | 1. Firm and elastic | Like extremely | 5 |
| | 2. Loss of elasticity | Like moderately | 4 |
| | 3. Moderately soft | Like slightly | 3 |
| | 4. Limp and floppy | Dislike | 2 |
| | 5. floppy | Dislike extremely | 1 |

FIGURE 4.1.16- SENSORY ANALYSIS OF MACKEREL FILLETS MARINATED WITH OLIVE OIL USING 5-POINT HEDONIC SCALE.

# DISCUSSION

## 4.2 DISCUSSION:

The negative effects of synthetic antioxidants on health have raised concerns regarding their use (Cordeiro et al., 2013; Gallego et al., 2013; Yang et al., 2016) therefore natural antioxidants are chosen over synthetic antioxidants since they are more dependable and have no adverse effects (Demir et al., 2014; Kapadiya et al., 2016).

Natural food additives are increasingly being used to enhance the flavor and shelf life of semi-preserved foods, including marinades (Ochrem et al., 2021). Therefore, this study depicts the use of one of these natural antioxidant that is kokum (*Garcinia indica*) that shows the antioxidant, anticancer and anti-ulcer properties (Yamaguchi et al., 2000) which prevents infections and is used as a preservative. The potent antioxidant present in kokum is Garcinol. However, no study has been conducted on the effect of kokum juice on mackerel. The main objective of the present study was to study the effect of kokum juice on biochemical, and sensorial quality of marinated mackerel fillet during refrigerated storage.

Sonavane et al., (2017) stated that the proximate composition of any fish is good indicator of quality of fish therefore in this study the proximate content of fish treated with kokum juice and olive oil marinades were studied.

## BIOCHEMICAL CONTENT IN MARINATED MACKEREL FILLETS-

4.2.1 Carbohydrate concentration in marinated mackerel fillets-

According to Kamalam and Panserat (2016), fish have negligible amount of carbohydrates compared to proteins and fats and still serve as an essential source for metabolic processes, growth and maintenance. Changes in the carbohydrate concentration of marinated samples are shown in Figure 4.1.2(B). Over the duration of the storage period, the concentration of carbohydrates in the samples treated with kokum juice and olive oil gradually dropped. Compared to the samples treated with kokum juice, those treated with olive oil exhibited a comparatively lower content of carbohydrates. Cervoni, 2022 reported that olive oil contains negligible amount of carbohydrates that is 1g per 100 g. Thakur, 2021 reported 14.3g per 100g carbohydrates in kokum fruit which correlated to my work. Kokum contains carbohydrates in the form of sugars and dietary fibres. On the other hand, olive oil is a fat therefore contains negligible amount of carbohydrates. Other researchers have also reported anti-obesity properties of kokum (Greenwood et al., 1981; Rao & Sakiriah, 1988). Indian mackerel and kokum are both naturally low in carbohydrates thereby making it suitable choice for individuals (Krishnamurthy et al., 1982; Shah, 2023). The decrease in carbohydrate concentration over time in kokum juice and olive oil is influenced by enzymatic reactions, fermentation, microbial activity, processing methods, and storage conditions.

#### 4.2.2 Amino acid concentration in marinated mackerel fillets-

Essential amino acids included in fish protein function as antioxidants. Amino acids play a role in texture, flavour, and overall product quality (Li et al., 2009). The concentrations of each sample declined drastically. Figure 4.1.3(B) shows that until the conclusion of the storage period, the concentration of amino acids was significantly higher in the samples treated with kokum juice than in the samples treated with olive oil as kokum is rich in essential as well as non-essential amino acids that includes garcinols and hydroxycitric acids that contributes to potential health benefits. Garcinol is also reported to show anti-cancer, anti-ulcer, anti-oxidative, antiglycation and antimicrobial activity. It plays important role in treatment of gastric ulcers (Nayak et al., 2010). Olive oil, being a fat-based product, does not contain as many amino acids because its primary constituents are fatty acids.

#### 4.2.3 Cholesterol concentration in marinated mackerel fillets-

Fish and fish products are rich in high-quality proteins and polyunsaturated fatty acids and they also contain significant amounts of cholesterol. Consuming fish products with high levels of cholesterol oxides may pose health risks. Monitoring and controlling cholesterol content during fish processing help ensure product safety and quality (Dantas et al., 2015). Figure 4.1.4 (B) demonstrates that cholesterol concentration in olive oil-treated samples grew during storage and was found to be quite high on the 21st day, but the cholesterol concentration reduced in samples treated with kokum juice. The cholesterol content showed variable levels in both the marinade samples studied. Raju, 2001 reported that Garcinia fruit lowers lipids such as cholesterol and triglycerides by triggering fatty acid oxidation. Kokum juice does not have the same effect on cholesterol levels as olive oil because it is not a fat-rich product. Instead, it provides additional health benefits like as antioxidant protection and potential weight management assistance.

4.2.4 Protein concentration in marinated mackerel fillets-

High-quality fish proteins offer the essential amino acids required for human health. Fish is regarded as one of the most inexpensive sources of protein, essential amino acids, and unsaturated fatty acids in human diets. When comparing marinades with olive oil, it was shown that kokum juice had the highest protein concentration as kokum contains 1.92% of protein as reported by Chate et al., (2019) while olive oil is not a significant source of proteins (Bilal et al., 2021). In agreement with our findings, Nisa & Asadullah (2012), Ali et al. (2013), Kumar et al. (2014) and Bahurmiz et al. (2017) estimated 15.1-22.4% protein in Indian mackerel. Over the course of the storage time shown in Figure 4.1.5 (B), a drop in the value of the protein content was noted for both of the samples treated with marinades. Because olive oil is predominantly a fat-based product derived from olives, its source means that it does not include substantial levels of proteins. Kokum juice and olive oil's progressive loss of protein concentration over time is caused by a variety of factors that can impact protein stability, including oxidation processes, microbial activity, storage conditions, and enzymatic reactions.

4.2.5 pH changes in marinated mackerel fillets-

Monitoring pH level helps in assessing the freshness and quality of fish. Fish with optimal pH levels are less likely to spoil quickly. Low pH impacts texture and taste whereas high pH can result in off-flavors. Fish products with an appropriate pH range inhibits the growth of harmful organisms which is crucial for food safety and shelf-life. Changes in the pH values of marinated samples are shown in Figure 4.1.1. In a study conducted by Kaya et al. (2023) on comparative effect of olive oil and rosehip sauce on the quality of marinated anchovies stored at $4^0$C resulted that pH of marinated anchovy increased during storage in parallel with time in both olive oil- and rosehip sauce-treated samples. In this study the pH of samples treated with olive oil increased during storage period whereas samples treated with kokum juice decreased during storage period. High significant differences were observed between olive oil and kokum juice treatments. Similar decreases were reported in previous studies (Sen & Temelli, 2003; Gokoglu et al., 2004; Maktabi et al., 2015). The pH of kokum juice is usually acidic ranging from 2-1 due to the presence of major organic acid namely hydroxycitric acid (HCA) which is found to be higher in proportion in kokum with a value of 22.80 and it has shown to significantly reduce body weight (Krishnamurthy et al., 1982). The low pH levels of kokum juice help to retard growth of spoilage microorganisms thereby promoting its shelf life.

4.2.6 Formaldehyde concentration in marinated mackerel fillets-

Jaman et al., (2015) reported that Formaldehyde occurs naturally in fish and seafood. As soon as fish undergo post mortem, trimethylamine oxide (TMAO) is broken down to dimethylamine and formaldehyde as its main product. TMAO is mainly found in marine fish (Jiang et al., 2006). Formaldehyde may be formed during the ageing and deterioration of fish flesh. This will eventually result in physical damage of fish or production of chemical metabolites such as biogenic amines or other unpleasant compounds (Gram et al., 2002; Arashisar et al., 2004). Now a days consumer is becoming more conscious of the application of formaldehyde in fish and also its side effects. During the storage period, a rise in the formaldehyde levels was noted in both samples. After 21 days of storage, it was discovered that samples treated with olive oil had higher formaldehyde concentrations as shown in Figure 4.1.6 (B). These preservatives have the potential to undergo chemical interactions that result in the formation of formaldehyde over time, especially if the fish samples are not maintained appropriately (i.e., exposed to heat, light, or air). Formaldehyde can form through various chemical reactions, including oxidation processes. When lipids in kokum juice or olive oil undergo oxidation, they can release formaldehyde as a byproduct therefore olive oil treated samples has higher formaldehyde concentration at the end of storage period. As per the studied reported by Aubourg, 1998; Orlick, Oehlenschlager, & Schreiber, 1991, protein denaturation and the loss of quality of fish have been associated with the formation of formaldehyde.

4.2.7 Thiobarbituric acid concentration in marinated mackerel fillets-

TBA analysis is an important quality index indicating fat oxidation. Connell, 1980 reported that oxidative rancidity is complex spoilage and especially occurs in fatty fishes therefore it is prone to occur in Indian mackerel. TBA value should be less than 3 mg malonaldehyde/kg in good quality material and not be more than 5 mg/kg. The limit level is reported as 7-8 mg/kg by Schormüller (1968 & 1969). Increases in TBA values have been reported for marinated anchovy (Gunsen et al. 2011), pacific saury (Sallam et al., 2007) and rainbow trout (Maktabi et al., 2015). TBA values are commonly used to measure the level of rancidity and are mainly related to the development of secondary oxidation products. In a study conducted by Kaya et al. (2023) on comparative effect of olive oil and rosehip sauce on the quality of marinated anchovies stored at $4^0$C resulted that the TBA values of all samples increased gradually during the 56 days of storage. Similar observation were noted in this study where the amount of thiobarbituric acid content rose in both samples during the storage period. As shown in Figure 4.1.7 (B), samples treated with kokum juice had higher thiobarbituric acid values. Fish samples treated with kokum juice and olive oil showed a rise in TBA value, which is indicative of lipid oxidation and the gradual formation of reactive compounds like MDA. Lipid oxidation can be reduced and product quality can be preserved with the use of appropriate handling, storage, and packaging techniques. TBA can have adverse effect on human health if consumed in large quantities over time. Kokum juice have low pH compared to olive oil which would influence chemical reactions during marination and affect lipid oxidation by increasing TBA values.

### 4.2.8 Sensory attributes for marinated mackerel fillets:

Decrease in the sensory scores of marinated anchovies with increase of storage period was also reported by Gokoglu et al. (2009), Topuz et al. (2014), Trabelsi et al. (2021). Kaya et al., (2023) reported all sensory properties demonstrated a decreasing trend with storage time. However, higher valuation in terms of appearance, odour, colour, and taste for the rosehip sauce-treated samp was observed compared to olive oil treated samples. Similar findings were reported in my study that the effects of kokum juice on the sensory properties of marinated mackerel fillets decreased significantly throughout the storage period. The sensory evaluation of marinated samples in terms of appearance, colour, odour, taste and texture was conducted by panellist. Although the panellists gave good score at the first evaluation of both samples after a period of time all sensory properties demonstrated a decreasing trend with storage time. However, high valuation in terms of appearance, colour, odour, taste and texture for kokum juice treated samples ($p < 0.05$) was observed compared to the olive oil treated samples. On the last day of storage (21$^{st}$ day) the kokum juice treated sample retained its good quality characteristics while sensory deterioration was observed in olive oil treated group. The results of present study demonstrated that kokum juice had positive effect on the sensory criteria on marinated mackerel fillets. Kokum juice has a unique tangy which can complement the natural taste of fish and can enhance the overall flavour of the fish dish, making it more appealing to sensory perception. On the other hand, olive oil has a distinct flavour that might not always pair well. The strong flavour of olive oil can sometimes overpower the delicate taste of fish, leading to a less favourable sensory experience for some individuals. Kokum juice, when used as a marinade, can help improve the texture of fish by tenderizing it slightly. The acidic properties of kokum juice can contribute to a tender and moist texture, which is often desirable in fish dishes. Other factors such as general appearance, odour collectively contribute to the overall sensory experience and perceived quality of the fish marinades.

# CONCLUSION

## 4.3 CONCLUSION-

In this study, analysis of biochemical and sensory parameters was carried out in mackerel fillets marinated with olive oil as control sample and kokum juice as experimental sample across a storage period of 21 days at $6^0$C. The procedures outlined in the standard protocols were followed for conducting the tests for the estimation of various biochemical components. Sensory analysis was carried out using 5-point hedonic scale. An extensive amount of variation was determined and observed in biochemical and sensory analysis of treated marinade samples during the course of study period. Kokum juice prevented the loss of biochemical and sensorial quality of marinated mackerel and it was observed that kokum juice was able to delay biochemical changes and has a positive effect on the appearance, color, texture, odor and taste of marinated fillets and extended their shelf-life. Thus, considering that the consumer preference is for natural anti-oxidants and preservatives, kokum juice being an indigenous natural anti-oxidant, could be used as a preservative, anti-oxidant and flavoring additive in fish marinades such as mackerel marinades. The study established the potential benefits of using kokum juice as a natural anti-oxidant in the preservation of different marinated seafood in order to satisfy both consumer preferences for natural additives and need of the food industry for effective preservation methods as it provides a healthy substitute for chemical preservatives when used as a fish preservative. Customers searching for organic and natural food preservation techniques may find this particularly appealing. The environment that kokum juice's acidity provides is not conducive to the growth of bacteria that cause spoiling. The fish's freshness is preserved to some extent by this acidity. Because kokum is less expensive than olive oil, consumers may tend to prefer it as a preservative.

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