Evaluation of Quality and Shelf Life of Tigertooth Croaker(*Otolithes ruber*) Fish Balls During Refrigerated Storage Condition

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Department of Fishing and Post-Harvest Technology

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This is to certify that we have examined the above Master's thesis and have found that it is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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The author

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List of Abbreviations

AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of Variance
FAO	Food and Agriculture Organization
DoF	Department of Fisheries
CFU	Colony Forming Unit
MT	Million Tonnes
TPC	Total Plate Count
TVB-N	Total Volatile Base Nitrogen

Abstract

The present study aimed to prepare ready-to-cook/fry fish ball from tigertooth croaker(*Otolithes ruber*) incorporating with various ingredients. Additionally, the quality and shelf life of prepared fish balls were investigated concerning sensory, chemical and microbial characteristics at 4°C after 7 days each. The experiment was conducted up to 21 days. The raw material exhibited good freshness and quality with a moisture content (78.86±0.23%), protein content (18.05±0.22%), lipid content (1.88±0.04%) and ash content (1.21±0.12%). After preparing surimi, four different types of fish balls were made using various ingredients at various amounts where 15% spices remain constant in all types of fish balls. The panelists evaluated the organoleptic qualities of the fish balls in terms of color, flavor, texture and overall acceptability. It was found that the fish balls treated with pea flour had excellent overall quality, while the fish balls treated with wheat and rice flour also showed good scores initially. The fish balls without any binding ingredients were deemed to be of poor quality. The organoleptic scores of all the types of fish ball slightly decreased within the storage period (21 days). The proximate analysis showed that fish ball without binding ingredients had higher moisture (77.95±0.10%) and protein content (14.04±0.08%) compared to others. In all types of fish balls the moisture content showed increasing trends whereas protein, lipid, and ash content showed decreasing trends within the storage period. TVB-N levels also increased significantly over time. Proliferation of microbes was observed during storage and TPC increased significantly on the 21st days of storage. The Total Plate Count(TPC) of fish balls increased significantly, reaching 2.84×10^6 CFU/g for fish ball with pea flour, 3.0×10^6 CFU/g for fish ball with wheat flour, 3.3×10^6 CFU/g for fish ball with rice flour and 3.51×10^6 CFU/g for fish ball without binding ingredients. Therefore, it revealed that fish ball with pea flour, fish ball with wheat flour and fish ball with rice flour were in acceptable condition up to 7 days of storage.

Keywords: *Otolithes ruber*, fish balls, quality, storage, shelf life

CHAPTER 1

INTRODUCTION

CHAPTER 1: INTRODUCTION

Fish is one of the most valuable commodities among various agricultural products. As a source of protein, fat and several vitamins, seafood especially fish has great nutritional potential. As fish is a perishable food commodity, decay is caused by enzymes, either from the fish itself or from microbes. Because of the increased water content, bacteria may proliferate and thrive more readily. Fish fat contains a lot of unsaturated fatty acids which can cause rancid odor by oxidizing fat content (Nurjanah et al., 2014). Therefore, it is essential to manage, process, and preserve fisheries goods with the goal of diversifying processed fishery products and extending their shelf life in addition to preventing damage to the fish. The croakers or jewfish are tropical demersal fish that live in fresh and salt water all over the world. They typically live at depths of less than 46 meters (150 feet) in sandy shorelines and estuaries. *Otolithes ruber* is a species of saltwater ray-finned fish that belongs to the croaker family, Sciaenidae. It is also referred to as the tigertooth croaker and the Indo-Pacific region is home to this species.

One of the fishery products that is heavily produced for additional processing in Asian nations is surimi. A refined form of fish flesh, surimi has unique technological properties that allow it to form gels and bind water and oils, making it a crucial component of many processed foods (Wasinnitiwong et al., 2022). Surimi is an intermediate product of minced fish that has been cleaned, refined and combined with cryoprotectants in order to achieve the right fish texture when cooked. Because of recent advancements in its manufacturing and use, surimi has emerged as one of the most popular fish paste products and a highly dynamic commodity in the Asian seafood market. Different kinds of surimi have been used as a source of protein in many nations to make surimi-based goods such as imitation crab sticks, fish cakes, fish balls, fish burgers, fish sausages and fish noodles (Park et al., 2013).

The process of planning, developing and launching a new product or refining an existing one to satisfy consumer demands, corporate objectives or industry trends is known as product development. Fish product development has gained increasing attention in both developed and developing nations due to the global demand for convenient, nutritious, and affordable food products. The desire for processed meals that are easier to handle, store, and prepare is rising quickly, as seen by current marketing trends (Pagarkar et al., 2011). In Southeast Asia, fish balls are the most commonly consumed products made from surimi. Along with surimi, the usual ingredients for fish balls in Japan and some other Asian nations include water, flour, sugar, salt, and monosodium glutamate (MSG). While some other countries employ a range of protein additives and spices in their fish ball formulation, those markets do not use any flavors or protein additives.

A product's quality is determined by how well it performs, how dependable it is, how long it lasts, and how much it costs overall. High-quality products are essential for building customer trust, ensuring satisfaction and fostering brand loyalty. Quality assessment of such processed food is very important for guarding consumers' health and hygiene. Due to the efficient removal of lipids and proteolytic enzymes, the kind and state of the washing process used during surimi manufacture have a significant impact on the quality of surimi-based goods (Priyadarshini et al., 2017).

1.1. Significance of the Study

According to FAO, (2020), vital amino acids, polyunsaturated fatty acids (PUFA), calcium, selenium, zinc, iodine, iron and vital vitamins A, B and D are all found in fish, which is a great source of high-quality protein with a high biological value (BV). It is known that, fish, as one of the primary sources of animal protein, is a perishable commodity due to the rapid onset of spoilage caused by bacterial activity and enzymatic reactions within the fish's body. Throughout the food supply chain, appropriate handling and processing methods are crucial to preventing spoiling and guaranteeing fish quality. By limiting and mitigating variables that cause quality deterioration and spoiling, these procedures seek to maintain fish quality and guarantee that it is fit for food until it reaches consumers (Andarwulan et al., 2011). By standardizing suitable, affordable, user-friendly and environmentally acceptable technology for turning such underutilized fish into delicious and profitable value-added meat products, the effective use of inexpensive fish in food might completely transform the fish business (Pagarkar et al., 2011).

A significant portion of fish landings in Bangladesh are caused by trash fish or inexpensive fish. In Southeast Asia, demersal fish species including croaker, threadfin bream, big-eye snapper and lizardfish that were formerly thought of as by-catch are mostly used in the manufacture of surimi. The majority of low-cost marine fishes

(LMF) in Bangladeshi waters are small mesopelagic or pelagic species, such as clupeids, scads, anchovies and small croakers (Nowsad and Hoque, 2009). Hossain et al., (2019) reported that the Bay of Bengal in Bangladesh currently receives over 0.1 million metric tons of marine underused species annually, with 50% of those species being shrimp by-catch. The majority of these by-catch are dumped into the ocean, while a small percentage are landed for human consumption. Both privately owned and government-operated shrimp trawlers dump thousands of metric tons of unwanted species each year. Utilizing these underutilized, discarded fish as a rich source of inexpensive animal protein by developing various products for human consumption could pave the way to address the growing nation's animal protein shortage and generate foreign exchange, as the population continues to grow faster than fish production (Ahmed et al., 2000). The people may be introduced to these fishes through items made from surimi. Research on the suitability of these readily available, inexpensive fish species, such as their capacity to form gel and other associated traits, is required to use them in the manufacturing of surimi.

Because of its abundance and market value, *Otolithes ruber*, also referred to as the tigertooth croaker, is regarded as an important species in the fisheries economy, especially in the Indo-West Pacific region. It is frequently caught using gillnets and bottom trawls and greatly contributes to the livelihoods of coastal fishing communities. The most crucial functional criteria of high-quality surimi-based goods is their capacity to form gel. Croaker surimi has been used to create high-quality items because of its well-known capacity to generate a robust, extremely elastic gel. The gel-forming ability is crucial in product development across various industries because it directly impacts the texture, functionality, stability and overall performance of the product.

Consumers, especially those in cities, are becoming increasingly interested in food items that come in ready-to-eat or ready-to-cook forms, like burgers, sausages and fish balls. The desire for processed meals that are easier to handle, store and prepare is rising quickly, as seen by current marketing trends. This condition is caused by several factors, including the growing number of working women, a reasonable rise in income, education and knowledge of freshness, quality, nutrition, hygiene and health, among others. As a result, numerous fisheries products with a variety of flavors, textures and looks have been developed (Pagarkar et al., 2011). Different value-added ingredients can alter the flavor of fish while also aiding in its preservation. As a result, people might enjoy the same thing in a variety of ways and to varying degrees (Chowdhury et al., 2017). Among them fish ball is one of them. According to Wang et al., (2022), fish balls are well-known ready-to-eat or fried items made from surimi that have had their bones removed and their fishy smells covered with a variety of spices and ingredients before being heated. For decades, they have been a popular snack in Bangladesh and other Asian nations (Zuraida and Budijanto, 2011). To assess the nutritional contribution of fish-based products, sensory and nutritional evaluations are necessary.

In this study, tigertooth croaker surimi was used as the primary ingredient for producing fish balls due to its excellent gel-forming ability, which is essential for achieving the desired texture in fish ball products. Given this quality, an effort was made to develop ready-to-cook/fry fish balls using tigertooth croaker. The objective of the study was to evaluate the quality of fish balls prepared from tigertooth croaker, focusing on their shelf life at 4°C. This evaluation included assessments of organoleptic, biochemical, and microbiological properties.

1.2. Objectives of the Study

1. To prepare fish balls from tigertooth croaker fish
2. To assess the changes of organoleptic characteristics and proximate composition of prepared fish balls during refrigerated storage condition
3. To determine the changes of Total Plate Count of prepared fish balls during refrigerated storage condition

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 2: REVIEW OF LITERATURE

2.1. Fisheries Resources of the Bay of Bengal

Through the resolution of maritime border disputes with neighboring states India and Myanmar, Bangladesh has been granted the right to 118,813 square kilometers in the Bay of Bengal, which includes its territorial sea and Exclusive Economic Zone. Considering the major river inlets and estuaries, which together make up a significant portion of the marine ecosystem, the total marine waters of Bangladesh are 121,110 square kilometer of which the shallow shelf sea and coastal waters make up roughly 35% and 20% of the total, respectively, with the remaining 45% located in deeper waters. One of Bangladesh's most vibrant and successful industries is fishing. It plays a crucial role in addressing protein deficiencies, providing jobs for jobless youth, facilitating foreign exchange, and boosting socioeconomic standing- the global debt accounts for 5.00% of national GDP and 30.81% of general agricultural GDP. In 2022–2024, fish and fish products accounted for 3.56% of total earnings, making them the second-largest exporters (Barua and Barua, 2024). But over time, inland fisheries have drastically decreased, and wetlands have also deteriorated. Overfishing and environmental degradation brought on by population oppression pose a threat to Bangladesh's fisheries (Barua et al., 2022).

Of the 214 million tons (MT) of fish produced in 2020, 157 MT were used directly for human consumption (FAO, 2022). Due to low-value discards, storage issues, short shelf life, and spoiling, 27% of all fish caught are typically wasted (FAO, 2022). In comparison to other animal protein sources, fish has the highest protein composition (about 57.2%), followed by beef (26%) and chicken meat (18.80%) (Wahyudi and Maharani, 2017). Because of their characteristics, fish with a high moisture content (around 80%) are more likely to deteriorate. Therefore, a processing step is needed to add value regarding flavor, aroma, texture, shape and nutrition.

2.2. Overview of Jewfish

One of the biggest groups of significant fishes that are harvested from Bangladesh's Bay of Bengal (BoB) water is croakers, also known locally as Jewfish or Poa mash (Sabbir et al., 2021). According to Fanning et al., (2019), twenty croaker species belonging to twelve genera have been identified in Bangladesh's marine waters. The most common genus of croakers is *Johnius*. The Belanger's croaker (*Johnius belangerii*), the Spindle croaker (*Johnius elongates*), the Bearded croaker (*Johnius dussumieri*), the Lesser tiger-tooth croaker (*Otolithes cuvieri*), the Pama croaker (*Otolithoides pama*), the Greyfin croaker (*Pennahia anea*), and the Spotted croaker (*Protonibea diacanthus*) are the most prevalent croaker species.

A study found that, one of the main coastal fisheries resources in tropical and temperate waters are croakers, also known as sciaenids (family Sciaenidae) (Liting et al., 2022). In addition, its species count and geographic range make it one of the most varied perciform families. With 289 recognized species and 69 genera, the Sciaenidae family is extensively distributed worldwide (Parenti, 2020). Another study found that there are 66 genera and 286 recognized species of Sciaenids worldwide (Froese and Pauly, 2022). Larger sciaenids and lesser sciaenids (small and medium sized) are the two basic categories into which Indian sciaenid species can be divided biologically. The Sciaenid family, which includes Tigertooth croaker(*Otolithes ruber*) is one of the species caught in large quantities as commercial by-catch and also by artisanal fishers. Bottom trawls, submersible gill nets, and angling fishing are the primary fishing techniques (Farkhondeh et al., 2018). Numerous biology studies have been conducted on *O. ruber* fish in various nations worldwide.

2.2.1. Distribution and Habitat of Tigertooth Croaker

The medium-sized tigertooth croaker(*Otolithes ruber),* is found in tropical, subtropical, and temperate waters, such as the Indian and Pacific Oceans, the Persian Gulf, the Oman Sea, China, and the Malayan archipelago (Brash and Fennessy, 2005). It is a fish that is indigenous to the Bay of Bengal and the Indian and Western Pacific Oceans. It can be found in brackish and marine environments over sand, mud, and rock substrates at depths ranging from 3 to 100 m (9.8 to 328.1 feet).

Figure 1: Tigertooth Croaker(*Otolithes ruber*)

2.2.2. Biology of Tigertooth Croaker

As juveniles, *Otolithes ruber* eat on planktonic crustaceans close to the surface, and as adults, they hunt fish and other benthic invertebrates. Throughout its distribution, *Otolithes ruber* is caught as bycatch and in mixed species fisheries. The landed fish is either sold fresh or preserved by salting or curing. It has a brownish upper body with diagonal black streaks, and a silvery lower body with a gold luster. As a carnivorous animal, croakers eat a variety of creatures, including small fish, mollusks, polychaetes and crabs.

2.3. Surimi Technology

2.3.1. Introduction of Surimi

In Japanese, the word "surimi" literally means "ground fish meat" and it is widely recognized that frozen raw fish proteins are utilized to make a variety of surimi seafood. East Asian cuisines frequently use surimi, a food item that comes in a variety of sizes and shapes, from fish balls to crab sticks and other types of seafood mimic. The majority of the many different types of fish that are currently used as raw materials in the manufacturing of surimi are marine fish. The first surimi manufacturing facility was established in 1994, marking the beginning of surimi's history in India in the 1990s.

The Indian business "Gadre Marine" rose to become the world's third-largest surimi maker, shipping to 24 nations. This demonstrates India's capacity to produce surimi and items derived from it. Although there is now less need for these products in domestic markets, their demand is growing. Since consumers are shifting to other options, these straightforward and healthful products have a lot of potential in Indian marketplaces. Around 250,000 MT of Alaska pollock surimi, 40,000 MT of other cold-water fish surimi, 650,000 MT of tropical fish surimi, 60,000 MT of fresh water fish surimi and 10,000 MT of surimi from other fish species were among the more than one million MT of surimi produced worldwide in 2020 (Gueneeugues, 2021).

2.3.2. Resources for Surimi Production

Fish species that are commercially viable for surimi production are distinguished by their volume abundance, price competitiveness and existing fishery situation (i.e., underutilized). Typically, almost 25% of the world's total surimi output comes from Alaska pollock, which is the most common cold-water white fish, mainly from the United States and Russia. The greatest fishery resources for surimi production are tropical fish species, which account for about 60% of the world's surimi production. These species include threadfin bream, lizard fish, big eye snapper, ribbon fish, croaker and others (Gueneeugues, 2021). Yan et al., (2021) stated that, in addition to the collected sea fish species, China produces more than 200,000 MT of surimi each year from the cultivated freshwater fish silver carp. A number of new fish species are being investigated as surimi raw materials as a result of the advancement of new technology.

2.3.3. Surimi Production and Quality

The main idea behind creating surimi is to take fish meat, remove any undesired parts (bones, scales, skin etc.), chop it, wash it and remove some of the water. During freezing and frozen storage, cryoprotectants are employed to prevent the denaturation of proteins. Given that freezing might result in denaturation and aggregation, this ingredient is necessary to guarantee the functional qualities of frozen surimi. One of the key features of premium surimi is its ability to gel, which allows it to mimic the texture of other seafood items. To produce high-quality surimi with a white color and a high gelling ability, fish mince is washed with a lot of fresh water during the production process to get rid of fat, water-soluble proteins (mostly heme proteins and proteases) and other contaminants (Park et al., 2013). Fish muscle proteins, more especially fish myofibrillar proteins, are refined during the surimi processing process while retaining an excellent gelling quality for frozen storage. Myofibrillar proteins, which include actin and myosin, are crucial for surimi gelation. The actomyosin ratio affects the cooperation of actin in gelation, even though myosin alone can create the gel. In gelation, myofibrillar proteins are denaturated by heat, which causes them to aggregate irreversibly and become cross-linked to create a three-dimensional network (Sun and Holley, 2011). Typical surimi processing stages include scaling (for tropical fish), heading, gutting, deboning, washing, dewatering, mixing with cryoprotectants, packaging and freezing.

Depending on the biological conditions of the fish, the ability of the muscle to create gel varies both within and within species. Age, season, sex, condition of death, freshness, fishing location etc. all contribute to diversity within the species. Numerous other characteristics, such as the mince's high fat content, the instability of the muscle proteins, the high proportion of dark to ordinary muscle, and the significant amount of sarcoplasmic protein, affect its ability to gel. A high muscle fat content reduces the gel-forming ability and prevents the production of mince products from non-fresh fish, even with the use of effective processing processes. Several intrinsic variables, including the presence of proteinases and transglutaminase, as well as the thermal denaturation and aggregation of muscle protein, typically have a significant impact on the quality of the surimi gel.

2.4. Surimi-based Products in Southeast Asia

Due to consumers' increasing health consciousness and desire for a diet rich in nutrients, the consumption of fish and fish products is increasing daily. At the moment, value-added mince and surimi-based fish products could satisfy customers' current nutritional needs and support Bangladesh's fish processing industry. As previously said, surimi is an intermediary product from which a wide variety of products can be created, contingent upon the inventiveness, ingenuity, and expertise of those involved in this line. Products made from surimi are well-liked in many nations, including the US, Canada, China, Japan, Vietnam, Malaysia and Thailand. These nations are home to numerous manufacturing plants that create surimi-based products for the international market. Overall, surimi products are a popular and versatile ingredient that can be used in a wide range of dishes. The quality and features of surimi and surimi products are influenced by fish species, harvesting conditions or season and the freshness of the fish mince. The heating method, temperature, and rate may all affect the quality of surimi products. Fish balls, imitation crab meat, fish sausage and breaded fish sticks are just a few of the many fish-based cuisine items that may be made with surimi, an affordable protein source. Products made from surimi have grown in popularity recently because of its distinctive texture, low fat, low cholesterol, and high nutritional content (Yousefi and Moosavi-Nasab, 2014).

2.4.1. Kamaboko

A type of classic Japanese seafood known as kamaboko, which is a homogenous protein gel, is used to symbolize a collection of traditional dishes made from the raw ingredients of surimi. It is also known as "fish cake." Fried kamaboko is known as Tenpura in West Japan and Satsuma Age in East Japan, whereas steamed kamaboko is known as Sumaki or Mushiita. Ryu et al., (2014) reported that kamaboko has high nutritional value. In essence, it is myofibrillar proteins in gel form, and the end products are tough and have a white look. The three fundamental procedures in processing kamaboko are washing, combining with salt, and heating. The elastic texture of kamaboko called ashi, is one of its distinguishing features.

2.4.2. Chikuwa

One of the surimi-based goods is chikuwa, which is made by seasoning, shaping with a bamboo rod and roasting at 130 to 180°C until the internal temperature reaches 75°C (Jia et al., 2018). Chikuwa is a type of gel-based fisheries product, and one crucial factor that might affect the product's quality is its texture. Low-quality chikuwa goods break easily when chewed. Consequently, the quality of chikuwa may be impacted by the fish meat used as raw materials to make surimi. The species of fish affect the texture of chikuwa (Cheng et al., 2014).

2.4.3. Fish Ball

According to Wodi et al., (2019), meatballs are a popular snack that appeals to people of all ages since they may be sold with a variety of ingredients and shapes to pique consumers' attention. Typically, beef, chicken, and fish are the meats utilized as raw ingredients to make meatballs. Grouper, snapper, tilapia, mackerel, and milkfish are among the fish used to prepare meatballs. According to Zamili et al., (2020), the community has so far produced meatballs out of the very costly fish tilapia (*Tilapia mariae*) and mackerel (*Scomber scombrus*). According to the National Standardization Agency (2017), fishballs are processed fisheries goods made from minced fish or at least 40% surimi combined with flour and seasonings, then formed and cooked. Fish balls are common ready-to-eat or fried items made from surimi that are made by removing bones and using heat to cover off fishy smells (Wang et al., 2022).

Fish ball is a fish-based product in Southeast Asia such as Taiwan, the Philippines and China. Fish balls are known by different names in each country: Look Chin Pla in Thailand, Yu Huan in Singapore, Bebola Ikan in Malaysia and Indonesia, Nga Soke in Myanmar, and Bola Bola in the Philippines. In Bangladesh and other Asian countries, they have been a common snack for many years (Zuraida and Budijanto, 2011). Fish balls are typically served fried, with soups, or with noodle meals. Fish balls have historically been made from underused freshwater and marine fish species. Making fish balls from Jewish fish will boost harvest value, improve utilization status, and lessen post-harvest loss from washing mince or preparing surimi. Therefore, both fisherman and the nation may gain from using the excess production of croaker for fisheries food products. According to several studies, fish balls are a significant value-added fish product made from mince or surimi (Affandi et al., 2019; Alkuraieef et al., 2020; Chowdhury et al., 2017). Fish balls are a well-liked and delicious value-added fish product among the "ready to eat" or "ready to cook" dishes (Dutta, 2009). Rapid urbanization, a rise in the number of working women (Akter et al., 2013) and rising consumer spending power have all contributed to a major shift in consumer tastes in recent years toward ready-to-eat foods (Hoque and Begum, 2016). To save time and energy, working people, as well as the younger generation of students and young adults, are becoming more interested in ready-to-eat foods (Akter et al., 2013). Regretfully, Bangladesh does not yet develop value-added fish products based on fish meat on a commercial basis. A lack of entrepreneurship, the shortage of raw materials, and the unpredictability of customer behavior make it impossible for Bangladesh to produce value-added fish products on a commercial scale, except of certain projects (Ejaz et al., 2009).

2.4.3.1. Fish Species Used for Preparing Fish Ball

Numerous studies have been carried out to create value-added fish products, such as fish balls, from freshwater species: rohu (*Labeo rohita*) (Dutta, 2009), striped catfish (*Pangasianodon hypophthalmus*) (Akter et al., 2013), common carp (*Cyprinus carpio*) (Abdel-Aal et al., 2014), tilapia (*Oreochromis mossambicus*) (Mugale et al., 2015) and marine water species: sea catfish (*Tachysurus thalassinu*s) (Nowsad et al., 2000), mosul bleak (*Alburnus mossulensis*) (Duman and Peksezer, 2016), Spanish mackerel (*Scomberomoru guttatus*) (Tee and Siow, 2017), Eastern little tuna (*Euthynnus affinis*) (Affandi et al., 2019), Indian mackerel (*Rastrelliger kanagurta*) (Alkuraieef et al., 2020). A fish ball was made by Hoque et al., (2007) using mince combined with sea cat fish (*Tachysurus thalassinus*), skipjack tuna (*Sarda orientalis*), horse mackerel (*Megalaspis cordyla*), jeweled shad (Ilisha filigera) and marine red jewfish (*Johnius argentatus*).

2.5. Importance of Studying Quality Evaluation

Fish deteriorates quickly. Fish spoilage happens instantly because of endogenous flesh enzymes. Nevertheless, at low temperatures, these enzymes become less active (Gandotra et al., 2012). Food preservation is necessary to extend its shelf life and maintain its flavor, texture, and nutritional content. As a result, food preservation techniques that are optimized must keep food from being harmed by microbes without sacrificing its nutritious content or quality (Ghaly et al., 2010).

To assess the nutritional contribution of fish-based products, sensory and nutritional evaluations are necessary. High-quality fish balls should be white, have a soft yet elastic feel, and not smell like fish. Fish balls and other surimi products must typically be transported and stored at or below -18°C, which results in high energy expenses. Additionally, proteins lose their biological value, digestibility and utilization when they are frozen and thawed (Yi et al., 2011). Lipid oxidation and microbiological growth have been identified as two significant variables contributing to the degradation of surimi products' quality and reduction in shelf life during refrigerated storage because of their unique handling and nutritional properties.

2.6. Storage Condition and Shelf Life of the Fish Ball

The shelf life of food is a crucial fact that needs to be understood. Food shelf life is measured by changes in sensory attribution, and microbiological, and chemical deterioration (Hashim et al., 2019). Furthermore, items made from surimi are susceptible to microbial contamination while being stored. Adding additional ingredients with a high antioxidant capacity is a feasible way to improve the nutritional qualities and shelf life of fish balls. Food must be preserved to extend its shelf life and maintain its flavor, texture, and nutritional content. Therefore, food preservation techniques must be adjusted to keep food from being harmed by microbes while preserving its nutritional content and quality. By creating unfavorable conditions that slow down microbial development and the metabolic breakdown of fish muscle, freezing can extend the shelf life of fish products (Gandotra et al., (2012). By creating unfavorable conditions that slow down microbial development and the metabolic breakdown of fish muscle, freezing can extend the shelf life of fish products (Gandotra et al., 2012). The majority of consumers favor fish products with a longer shelf life and higher nutritional content, and freezing makes it difficult to maintain the freshness of fish (Viji et al., 2015). The shelf life of fish balls stored in unrefrigerated, unprotected bamboo baskets is only two days.

2.7. Microbiological Status of Surimi-based Products

The microbiological quality of fisheries products can be impacted by the implementation of sanitation and hygiene requirements throughout processing. Bacteria like *Pseudomonas*, *Vibrio*, *Serratia*, *Listeria* and *Micrococcus* frequently cause the microbiological growth of fish and fish products, which leads to fish deterioration, decreased nutritional value, and decreased sensory scores (Abdollahzadeh et al., 2016). The Total Plate Count (TPC) of fish balls refers to the total number of viable (live) bacteria in a given amount of the product, typically measured in colony-forming units per gram (CFU/g). It is a standard microbiological test used to assess the overall bacterial load, which can indicate freshness, hygiene, and potential spoilage. The internationally recommended microbiological safety criteria assigned to ready-to-eat fishery products is also at 6 log CFU/g (ICMSF 1986). The main disadvantage of fish ball products is that they quickly decompose if they are not handled properly. Besides the role of putrefactive bacteria, pathogenic bacteria can easily contaminate fish ball products if the handling during processing and distribution is not carried out hygienically. Due to the comparatively short shelf life of fish ball 4-5 days at about 5°C storage, the products are designed for immediate delivery. According to Alkuraieef et al., (2020), reducing the humidity and increasing the amount of filler material (rice, potatoes) can also inhibit microbial growth by lowering the water activity required for bacterial growth and decreasing the protein percentage. The addition of additional ingredients, such as spices and garlic, increases the inhibitory effectiveness of these ingredients against the growth of numerous bacteria.

CHAPTER 3

MATERIALS AND METHODS

CHAPTER 3: MATERIALS AND METHODS

3.1. Collection of Raw Materials

Fish samples were collected from Sabrang Bazar, local market, Teknaf, Chattogram (20°50′1″ N and 92°18′25″ E), Bangladesh. Collected samples were packed carefully in polythene bags, transported in an insulated box with ice layers, and brought to the Nutrition and Processing Lab at Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh.

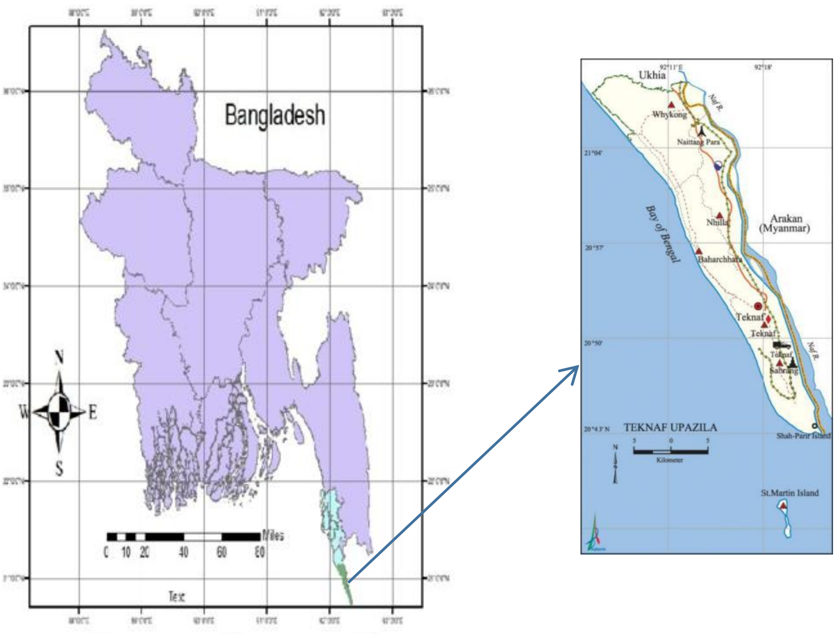


Figure 2: Study area map

3.2. Determination of the Proximate Composition of Raw Fish

3.2.1. Sample Preparation

The collected samples were ground for proximate composition analysis in the laboratory. Three replications were used during the determination of proximate composition for each sample. Samples were analyzed according to AOAC methods (AOAC, 2012).

3.6.2. Determination of Moisture

The idea is that when the sample is dried in an oven to a fixed weight, the moisture is measured by the weight decrease. In this study, moisture content was determined by oven drying method (AOAC, 2012). At first, the empty crucible was sterilized into hot air oven. The empty crucible was then cooled, and the weight of the empty crucible was taken. Then, about 3 g of each sample was weighed and put into a crucible. Then, the sample with crucible was placed into the chamber of the dry oven, and set time for overnight (12 hours) at 105. After that, the sample was put into a desiccator up to cooling. After cooling, the final weight of the sample with the crucible was taken, and moisture content was determined by using the following formula-

$$\text{Moisture content (\%)} = \frac{\text{Weight of wet material(g)} - \text{Weight of dry material (g)}}{\text{Weight of wet material (g)}} \times 100$$

Figure 3: Hot air oven

3.6.3. Determination of Lipid

Lipid content was determined by using Soxhlet apparatus (Model: RD 40, Food ALYT) recommended by AOAC (2012). At first, sterilization of empty beaker was done into hot air oven and switch on the chiller 1 and then 2. The work was started when the temperature got below 12°C. Then weight of the empty beaker was taken and marked the beaker. After that, about 2g of each ground sample was taken with the help of foil paper and then taken into thimble paper. The thimble paper was set under the magnetic holder by a magnetic ring and lifted it up. Then, 70ml of diethyl ether was taken in the marked beaker and screwed the beaker with solvent under the condenser and opened the stopcock vertical position. The solvent was heated until it boiled, allowing the vapor to rise into the condenser, where it cooled and condensed back into liquid form, dripping over the sample. This process enables the acetyl ether to wash over the solid sample repeatedly, extracting the lipids through continuous recycling of the solvent over several hours. Once extraction was complete, the solvent containing the dissolved lipids was collected for further analysis. This method is essential for assessing the nutritional value and quality of food products, and the results are typically documented and illustrated in Figure 4, showcasing the Soxhlet apparatus setup and extraction process.

Formula for determination of lipid:

$$\text{Lipid content (\%)} = \frac{\text{Weight of lipid (g)}}{\text{Weight of sample (g)}} \times 100$$

Figure 4: Lipid content determination

3.6.4. Determination of Protein

The crude protein of the fish was determined by Micro-Kjedahl method (AOAC, 2012).

a) Digestion compact system (DK 20/26, VELP scientifica)

b) Distillation system (Model: UDK 129, VELP scientifica)

Protein determination was done by following three different steps-

i. **Digestion:** Initially, 4g of catalyst and 5ml of concentrated H_2SO_4 were added to the digestion tube containing roughly 0.3g of ground material. After that, the tube was inserted into the digestion unit and allowed to digest for half an hour. After digestion, the digestion tube was cooled at room temperature for 30 minutes and then 25mL distilled water in the digestion tube.

ii. **Distillation:** After that, 10ml mixed indicator was taken in the conical flask of the distillation unit, and taken 25ml NaOH (white pipe) and distilled water (black pipe) were in the below pipe of the distillation unit.

iii. **Titration:** Finally, the sample was titrated with 0.2N HCl.

Total nitrogen was calculated by using the following formula-

$$\text{Nitrogen (\%)} = \frac{(\text{A}-\text{B}) \times \text{Normality of acid} \times \text{Milli equivalent weight of } \text{N}_2 \text{ (0.014)}}{\text{Weight of sample (g)}} \times 100$$

Where,

A= ml of titrant of sample

B= ml of titrant of blank

Percentage (%) of crude protein = Nitrogen (%) × 6.25

Digestion unit **Distillation unit** **Titration unit**

Figure 5: Protein content determination

3.6.5. Determination of Ash

Ash content was determined by ignition of samples in a muffle furnace (Model: LHMF 100A, LABNICS Equipment) (AOAC, 2012). Firstly, the empty crucible was sterilized into a hot air oven and then cooled the empty crucible and taken weight of empty crucible. Then, about 3g of each ground sample was taken into a pre-weighed crucible and placed into the chamber of muffle furnace for 5hours at 550^{o}C. After that, the sample with the crucible was placed in the desiccator and weighed. Finally, the percentage of ash content was calculated by using the following formula-

$$\text{Ash content (\%)} = \frac{\text{Weight (g) of ash}}{\text{Weight (g) of sample}} \times 100$$

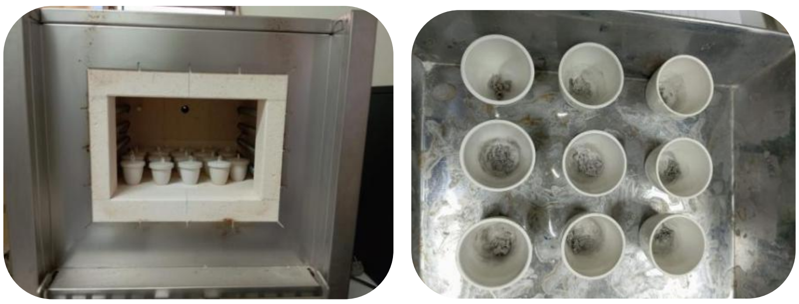


Figure 6: Ash content determination

3.3. Preparation of Surimi

Surimi production began with the process of eviscerating and cleaning the fish from all types of dirt. The head and entrails of the fish were removed and washed. Then the removal of scales and the separation of fish meat was done mechanically using a meat bone separator. The resulting mashed meat was dark in color because it still contained fat, residual blood and other sarcoplasmic proteins. The mashed meat was then washed to separate these components, using clean cold water with a volume of water 4 times the volume of mashed meat. During washing, NaCl was added to facilitate the removal of water at a later stage. The water that was still contained in the surimi after washing was removed as much as possible by pressing the mashed meat in a filter cloth. After that cryoprotectants (4% Sucrose, 4% Sorbitol) were used to inhibit the protein denaturation process during freezing and frozen storage. This surimi was packed in polythene bag and stored in cold storage at -18°C for further use.

The step-by-step surimi production procedure is shown below-

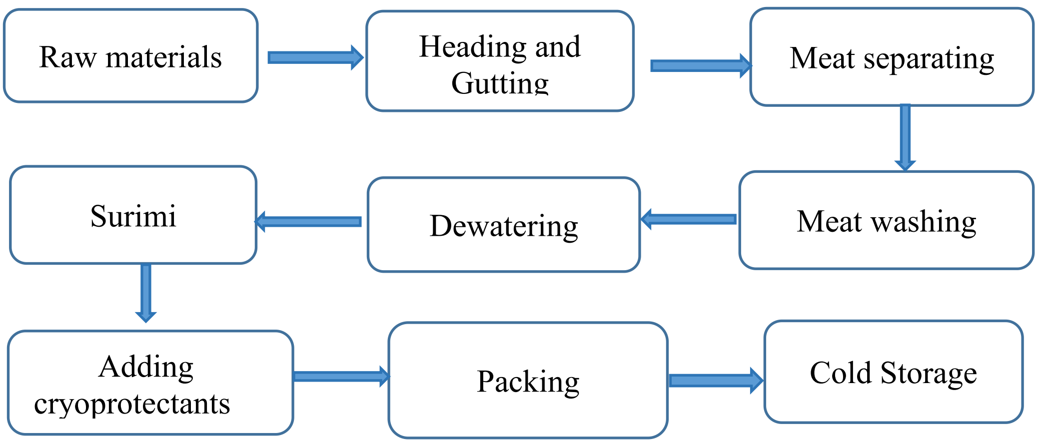


Figure 7: Procedure of surimi production (Panpipat et al., (2023))

Figure 8: Meat bone separator

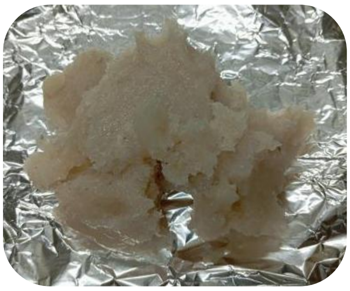
3.4. Preparation of Fish Balls

Four different types of fish balls were made. The percentage of spices in each sample was 15% and remained constant throughout. All the ingredients were weighed accurately showed in Table 1. Firstly, 60% surimi (150g) and required amount of salt were taken in a mixture and mixed for 5 minutes. To make dough, more weighed components, the necessary amount of water, and salt were added after five minutes. The dough was prepared and formed into small balls. For 10-15 minutes, the prepared fish balls were submerged in boiling water until they floated.

Table 1: Level of ingredients and their percentage (%) used for different fish balls preparation

Ingredients	Fish ball with pea flour	Fish ball with wheat flour	Fish ball with rice flour	Fish ball without ingredients
Surimi	150g	150g	150g	150g
Onion	15g	15g	15g	15g
Garlic	10g	10g	10g	10g
Ginger	7.5g	7.5g	7.5g	7.5g
Salt	7.5g	7.5g	7.5g	7.5g
Chilli	2g	2g	2g	2g
Egg white	20ml	20ml	20ml	20ml
Oil	5ml	5ml	5ml	5ml
Cumin	0.75g	0.75g	0.75g	0.75g
MSD	0.75g	0.75g	0.75g	0.75g
White pepper	1.25g	1.25g	1.25g	1.25g
Pea flour	63g	-	-	-
Wheat	-	63g	-	-

flour				
Rice flour	-	-	63g	-



1. Surimi

2. Mixing with ingredients

3. Shaping into fish ball

4. Boiling

5. Cooling

6. Fish ball

Figure 9: Procedure of fish ball preparation

3.5. Packaging and Storage of Prepared Fish Balls

After preparing fish balls, the products were cooled properly to maintain their shape and stored to maintain their quality and safety. Firstly, boiled fish balls were allowed to cool down using ice to prevent the loss of their original texture. After that, the water from the fish balls was removed by using clean tissue paper and then they were carefully transferred into an airtight polythene bag. After sealing the bag properly, fish balls were kept in chilled storage (4°C) to study the shelf-life attributes. The storage studies included sensory, biochemical and microbiological evaluation of four different types of fish balls at 7-days of intervals of 21 days.

Figure 10: Packaging of fish balls for storage

3.6. Evaluation of Quality and Shelf Life of the Prepared Fish Balls

3.6.1. Sensory Analysis

A ten-person panel of teachers, students, and employees from Chattogram Veterinary and Animal Sciences University's Department of Fishing and Post-Harvest Technology evaluated the items' sensory qualities. Scores were used from 10 to 1.

Table 2: Scoring table for color test using sensory method (Shikha et.al., 2019)

Score	Description	Comment
8 to 10	Contents appropriately colored (bright brown)	Excellent
6 to 7	Contents generally acceptable colored (brown/white)	Very good
4 to 5	Contents moderately colored (grayish)	Good
1 to 3	Contents considerably discolored (dark gray)	Poor

Table 3: Scoring table for flavor test using sensory method (Shikha et.al., 2019)

Score	Description	Comment
8 to 10	Contents have no abnormal odor and have a very good characteristic flavor and seasoning	Excellent
6 to 7	Contents have no abnormal odor, flavor and seasoning, satisfactory flavor	Very good
4 to 5	Contents have slightly raw or scorched odor or flavor; seasoning seems to be somewhat inadequate	Good
1 to 3	Contents have strong abnormal odor and a poor flavor	Poor

Table 4: Scoring table for texture test using sensory method (Shikha et.al., 2019)

Score	Description	Comment
8 to 10	Hard, highly elastic	Excellent
6 to 7	Moderately hard and elastic	Very good
4 to 5	Slightly soft and elastic	Good
1 to 3	Soft and broken, no elasticity	Poor

Figure 11: Sensory analysis of prepared fish balls

3.6.2. Biochemical Analysis of the Prepared Fish Balls

3.6.2.1. Assessment of Proximate Composition

The proximate composition of fish balls, including their moisture content, ash content, crude protein content, and crude lipid content, was examined. The composition was examined following the Association of Official Analytical Chemists' standard operating procedure (AOAC, 2012).

3.6.2.2. Determination of Total Volatile Base Nitrogen (TVB-N)

According to the standards set by the Association of Official Analytical Chemists (AOAC, 2012), estimating total volatile bases-with a special emphasis on volatile nitrogen compounds is a crucial analytical process used to evaluate the quality and freshness of food. First, 25g of the sample was weighed and put into a conical flask with 50ml of 7.5% trichloroacetic acid (TCA), which aids in the extraction of volatile nitrogen compounds. After homogenizing the mixture, the liquid extract was separated from the solid residues using Whatmann filter paper. A second conical flask was filled with 25 ml of 4% boric acid and a few drops of methyl red indicator after 25 ml of the filtered extract was moved to a distillation tube. To establish alkaline conditions and enable the distillation of volatile bases into the boric acid solution, 30 milliliters of 10% sodium hydroxide (NaOH) was added throughout the three-minute distillation process. Following distillation, sulfuric acid (H_2SO_4) was added to the resultant solution until the methyl red indicator changed color, indicating that the reaction had reached its endpoint. The amount of sulfuric acid used is then utilized to compute the total volatile nitrogen.

Total Volatile Base Nitrogen was calculated by using the following formula-

TVB-N (mg/100g) = $\frac{V \times N \times 100 \times 14}{W}$

Where,

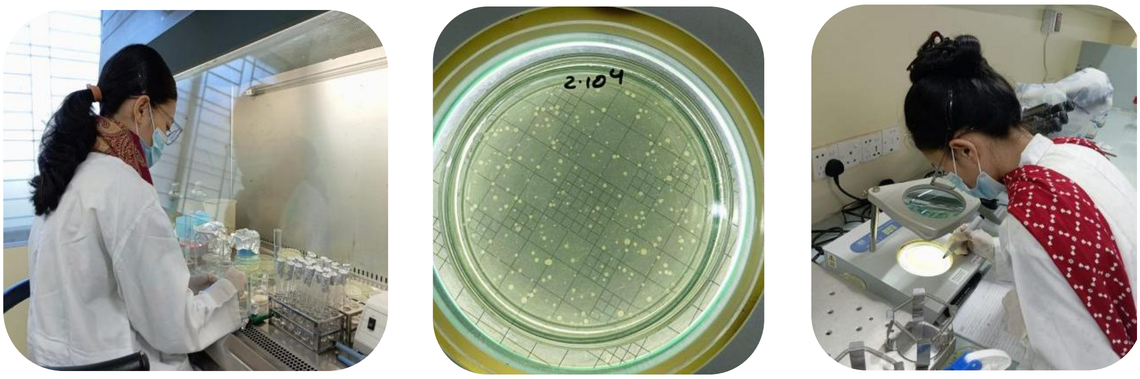
V=volume (ml) H_2SO_4 used for the sample.

N=normality of H_2SO_4.

W= Weight of sample.

3.6.3. Total Plate Count of Fish Balls

10g of the fish ball was first aseptically weighed and combined with 90ml of physiological saline in a homogenizer to create a homogenous sample. 9ml of physiological saline was used to create serial decimal dilutions in order to get ready for bacterial counting, which produced serial dilutions of 10^1, 10^2,10^3,10^4,10^5 and 10^6. For the spread plate technique, 0.1 ml of inoculum from each dilution was poured onto Plate Count Agar (PCA) plates and dispersed evenly with sterile glass bits. Then the plates were incubated at 37°C for 48 hours to allow bacterial colonies to develop. All microbiological tests results were expressed as the colony forming units per gram (CFU/g).



Sample preparation **Bacterial colony** **Colony counting**

Figure 12: Total Plate Count of the prepared fish balls

3.7. Statistical Analysis

Analysis of variance was performed on the collected data (ANOVA). A difference was considered significant if it was less than 0.05. The SPSS software, version 30.0.0(172), was used to perform statistical analyses.

3.8. Overview of Research Methodology

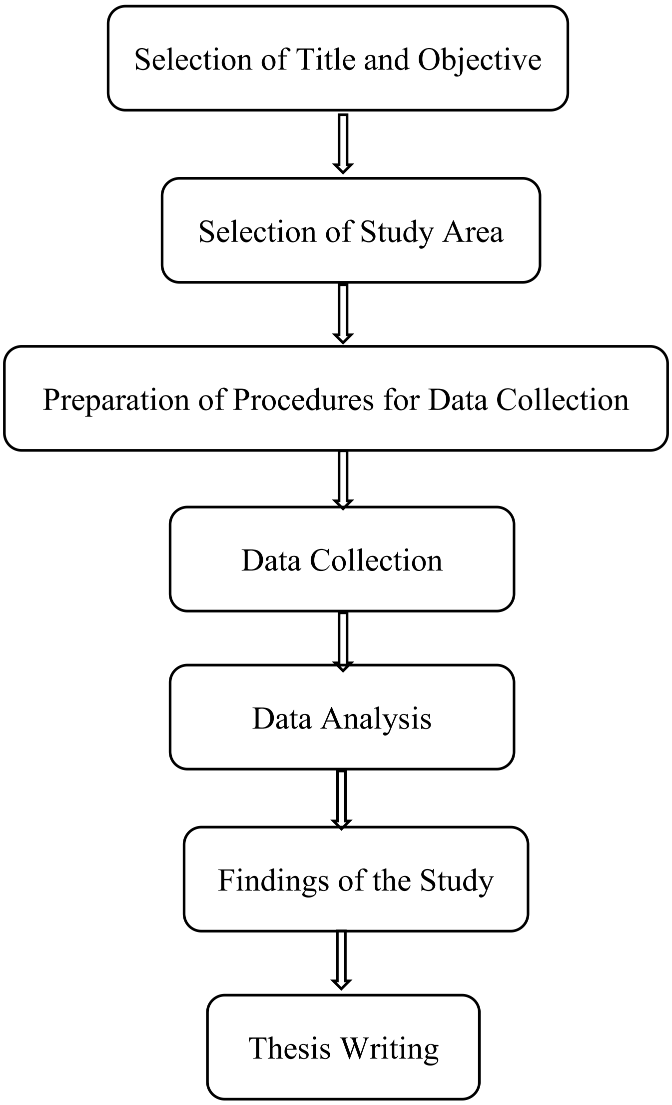


Figure 13: Framework of research methodology

CHAPTER 4

RESULTS

CHAPTER 4: RESULTS

4.1. Analysis of the Morphological and Biochemical Composition of Raw Fish

The overall quality of tigertooth croaker(*Otolithes ruber)* fish used in this study as raw materials was good freshness, characterized by a good appearance, a natural fishy odor and a firm, elastic texture. The fish had 78.86±0.23% moisture, 18.05±0.22 % protein, lipid at 1.88±0.04% lipid and 1.21±0.12% ash.

Table 5. Morphological characteristics of tigertooth croaker (*Otolithes ruber*)

Characteristics	Raw fish
Total length (cm)	22.57±0.45
Standard length (cm)	19.43±0.4
Weight (g)	241.67±7.64
Organoleptic characteristics	Color is silvery, natural fishy odor, firm and elastic
Freshness	Good

The mean values of three replications ± SD are used to represent all the values.

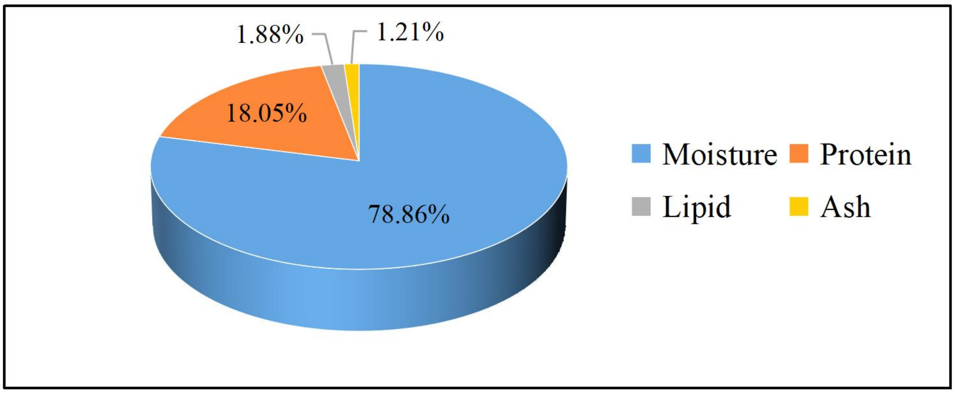


Figure 14: Proximate composition of raw fish

Chapter 4: Results

4.2. Evaluation of Quality and Shelf Life of Prepared Fish Balls

4.2.1. Sensory Analysis During the Storage Period

The sensory evaluation of prepared four different types of fish balls was evaluated by a testing panel to assess the consumer's acceptability. During storage at 4°C, it was discovered that the various ingredients affected the fish balls' color, flavor, texture, and general acceptance. Table 6 displays the average results for the various fish balls' color, flavor, texture, and overall acceptance. The fish balls prepared with pea flour at 4°C temperature exhibited significant deterioration, with color scores declining from 8.67 to 7.28 which changes bright brown to light brown over time, flavor falling from 8.80 to 2.03 which experienced a very good characteristics flavor to strong abnormal and poor flavor by day 21 and texture dropping from 8.07 to 6.23 which converts hard, highly elastic texture to moderately hard texture. Overall quality decreases from 8.51 to 4.04.

Similarly, the fish balls containing wheat flour maintained higher scores, starting at 8.33 for color and ending at 7.12, 8.1 to 1.8 for flavor, and 8.6 to 6.03 for texture with overall acceptability of 8.34 to 4.01 across the storage period. Fish balls treated with rice flour showed also good scores initially, and the values also decreased with the storage period. On the other hand, the sensory evaluation scores of fish balls without binding additives were generally lower than those of other samples. Therefore, the overall acceptability of fish ball containing pea flour was more preferable to the panelists among the other samples at a 5% level of significance. During the storage period, all attributes of all samples decreased significantly.

Table 6: Results of the sensory analysis during storage

Sample	Storage time (Days)	Sensory attributes			
		Color	Flavor	Texture	Overall acceptability
Fish ball with pea flour	0	8.67±0.58^{a}	8.80±0.20^{a}	8.07±0.06^{a}	8.51±0.27^{a}
	7	8.47±0.06^{b}	8.10±0.10^{b}	7.93±0.06^{b}	8.17±0.06^{b}
	14	7.36±0.21^{c}	2.93±0.06^{c}	6.93±0.06^{c}	5.54±0.11^{c}
	21	7.08±0.06^{d}	2.03±0.06^{d}	6.10±0.11^{c}	4.04±0.04^{d}

Fish ball with wheat flour	**0**	8.33±0.29^{a}	8.1±0.10^{a}	8.6±0.10^{a}	8.34±0.16^{a}
	7	8.07±0.06^{b}	8.07±0.06^{b}	8.07±0.06^{b}	7.72±0.05^{b}
	14	7.21±0.10^{c}	2.77±0.06^{c}	6.93±0.06^{c}	5.53±0.07^{c}
	21	7.02±0.26^{d}	1.8±0.10^{d}	6.03±0.06^{d}	4.01±0.14^{d}
Fish ball with rice flour	**0**	8.53±0.06^{a}	8.03±0.06^{a}	8.13±0.06^{a}	8.3±0.03^{a}
	7	8.07±0.06^{b}	7.97±0.06^{b}	8.03±0.06^{b}	8.02±0.04^{b}
	14	7.23 ±0.10^{c}	2.77±0.06^{c}	6.83±0.15^{c}	5.5±0.10^{c}
	21	7.02±0.26^{d}	1.8 ±0.06^{d}	5.97±0.06^{d}	4.02±0.14^{d}
Fish ball without ingredients	**0**	6.57±0.12^{a}	4.93±0.06^{a}	4.43±0.06^{a}	5.48±0.08^{a}
	7	6.07±0.06^{b}	4.14±0.13^{b}	4.23±0.06^{b}	4.81±0.08^{b}
	14	5.73±0.06^{c}	2.93±0.06^{c}	3.97±0.06^{c}	3.98±0.02^{c}
	21	5.47±0.15^{d}	1.1± 0.06^{d}	3.52±0.06^{d}	3.92±0.05^{d}

The mean values of three replications ± SD are used to represent all the values. Tukey-HSD test, one-way ANOVA, and different superscripts (a-d) in the same row indicate significant differences in the sensory characteristics of the different samples ($p<0.05$).

4.2.2. Biochemical Analysis of Prepared Fish Balls During the Storage

4.2.2.1. Analysis of Proximate Composition

The data (Table 7) provided a detailed analysis of the changes in the proximate composition of prepared fish balls.

The biochemical composition of all fish ball samples focusing on moisture, protein, lipid and ash content stored at 4°C over 21 days showed a significant difference. The fish balls prepared with pea flour, moisture content increased from 69.36% to 70.99%, while protein levels declined from 12.51% to 12.06%. Lipid and ash content also decreased from 3.08% to 2.54% and 1.92% to 1.13%. Similarly, the moisture content of the other three samples showed an increasing trend over the storage period. Conversely, the protein, lipid and ash content decreased gradually over the 21-day storage period.

Table 7: Results of proximate composition analysis during storage

Sample	Storage time (Days)	Proximate composition			
		Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Fish ball with pea flour	0	69.3±0.03^{d}	12.5±0.03^{a}	3.08±0.04^{a}	1.92±0.05^{a}
	7	69.9± 0.05^{c}	12.2±0.02^{ab}	2.98±0.01^{a}	1.49±0.03^{b}
	14	70.5±0.11^{b}	12.2±0.02^{bc}	2.75±0.04^{b}	1.24±0.04^{c}
	21	70.9±0.06^{a}	12.0±0.05^{c}	2.54±0.04^{c}	1.13±0.04^{c}
Fish ball with wheat flour	0	70.5±0.04^{d}	11.4±0.05^{a}	3.69± 0.04^{a}	1.95± 0.05^{a}
	7	71.0±0.04^{c}	11.2±0.04^{b}	3.59± 0.02^{b}	1.68± 0.04^{b}
	14	71.7±0.08^{b}	11.1±0.05^{c}	3.43± 0.02^{c}	1.26± 0.04^{c}
	21	72.1±0.06^{a}	10.9±0.04^{d}	3.29±0.02^{d}	1.15±0.04^{c}
Fish ball with rice flour	0	69.7± 0.12^{d}	11.8± 0.06^{a}	3.77± 0.08^{a}	1.85± 0.04^{a}
	7	70.3± 0.12^{c}	11.7± 0.09^{b}	3.52±0.04^{b}	1.75±0.03^{b}
	14	70.7±0.09^{b}	11.6±0.04^{b}	3.35±0.05^{b}	1.64±0.02^{c}
	21	71.3±0.05^{a}	11.4±0.03^{c}	3.13±0.02^{c}	1.48±0.02^{d}
Fish ball without ingredients	0	77.9±0.10^{d}	14.0±0.08^{a}	2.39±0.04^{a}	1.61±0.04^{a}
	7	78.6±0.65^{c}	13.9±0.08^{b}	2.13±0.05^{b}	1.53±0.03^{ab}
	14	79.1±0.11^{b}	13.7±0.09^{bc}	1.88±0.02^{c}	1.47±0.03^{bc}
	21	79.7±0.06^{a}	13.6±0.09^{c}	1.51±0.01^{d}	1.39±0.01^{c}

The mean values of three replications ± SD are used to represent all the values. Tukey-HSD test, one-way ANOVA, and different superscripts (a-d) in the same row indicate significant differences in the proximate composition of the different samples ($p<0.05$).

4.2.2.2. Determination of TVB-N Content of Prepared Fish Balls

The TVB-N values of all treatments were found to be significantly increased with the storage period shown in Figure 15.

The TVB-N value of fish ball using pea flour was 2.77±0.04 initially, as time progressed, it increased gradually and after 21 days of storage, it reached 7.79±0.04. The TVB-N value of fish ball containing wheat flour was 2.49±0.01 initially, and it reached to 7.95±0.01 after 21 days of storage. For the fish balls treated with rice flour, the TVB-N value was 2.33±0.03 initially, which was up to 7.7±0.03 after 21 days of storage. The lowest TVB-N value was found in fish balls without binding ingredients initially at 1.65±0.03 and it touched the value of 5.53±0.02 after 21 days of storage. The data were placed in Figure 15.

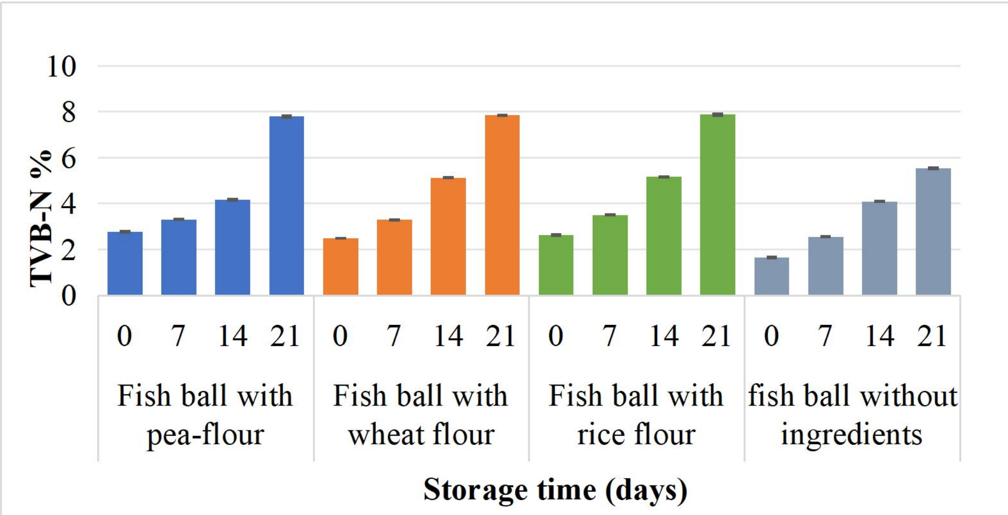


Figure 15: Changes of TVB-N content of prepared fish balls during storage period

4.2.3. Total Plate Count of Prepared Fish Balls During Storage

The values of Total Plate Count (TPC) shown in Figure (16) of all types of fish balls were found to be significantly increased with the storage period.

To track the microbiological load in four distinct kinds of fish balls while they were being stored at 4°C, the Total Plate Count was calculated every seven days. Initially the lowest value of Total Plate Count was found in fish ball with pea flour. The value was ranged from 1.09×10^4 to 2.84×10^6 CFU/g during the 21-day storage period. The value of Total Plate Count in fish balls made with wheat flour recorded 1.17×10^4 CFU/g to 3.1×10^6 CFU/g. The fish ball containing rice flour obtained the Total Plate Count from 1.22×10^4 CFU/g to 3.3×10^6 CFU/g after 21 days of storage. For the fish ball without binding ingredients, the value was 2.8×10^4 CFU/g and ended with 3.51×10^6 CFU/g.

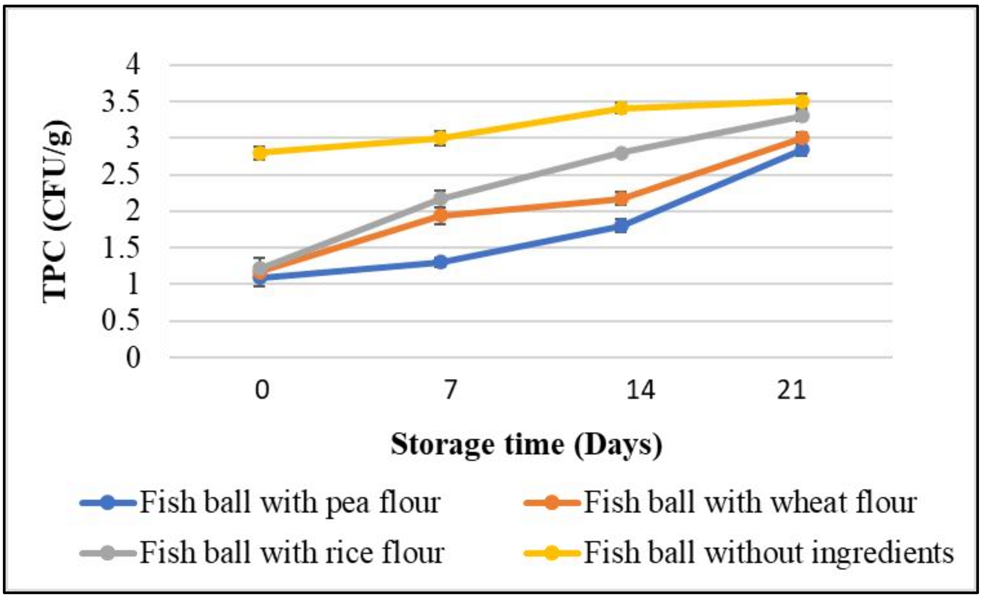


Figure 16: Changes of TPC of prepared fish balls during storage period

Therefore, microbiological examination, biochemical composition and sensory quality features showed that the fish balls were in acceptable conditions for 7 days and started deteriorating onward.

CHAPTER 5

DISCUSSIONS

CHAPTER 5: DISCUSSIONS

5.1 Analysis of the Morphological and Biochemical Composition of Raw Fish

In general, raw material freshness is crucial since long-term storage before freezing lowers fish muscle's product quality and storage stability. The assessment of tigertooth croaker fish signified a number of important quality and freshness indicators that were essential to customer satisfaction and safety. The fish's average length and weight were in line with what was expected for its species. A firm texture and a silvery appearance were indicative of acceptable quality. Fish quality, nutritional value, physiological condition and habitat are all predicted by the chemical makeup of their flesh (Ravichandran et al., 2011). Proximate analysis confirmed its nutritional value, revealing a moisture content of 78.86%, protein level of 18.05%, lipid content of 1.88% and ash content of 1.21% (Likhar et al., 2022).

5.2. Evaluation of Quality and Shelf Life of Prepared Fish Balls

The quality and shelf life of the jew fish balls were determined by comparing the people acceptance and changes in biochemical and microbial load of those fish ball after production and after 21 days of production.

5.2.1. Sensory Evaluation

The most reliable test for processed fish products is sensory evaluation, which can be used to create a substantial-quality index. Panel acceptability is correlated with differences in sensory qualities.

This study provided a significant data about how the sensory characteristics were altered during 4°C storage temperature. In the opinion of the panelists, fish ball with pea flour, wheat flour and rice flour had a pleasing brown appearance initially that was bright and shiny overall. The fish balls without binding ingredients did not show good scores for organoleptic characteristics may be due to not using any binding agents. When it came to texture, the fish balls made with wheat flour were hard and highly elastic followed by the fish ball made with pea flour, fish balls made with rice flour than the ones made without binding ingredients. The texture of the fish ball without binding ingredients was nonetheless firm and elastic. As per Pramuditya and Yuwono, (2014), the fish balls' content, production method and heating duration, might impact their texture. The results obtained from the present study are more or less in agreement with Yi et al., (2011) where meat products' look and color scores declined as storage times for Collichthys fish balls increased.

5.2.2. Biochemical Analysis of Prepared Fish Balls

5.2.2.1. Assessment of Proximate Composition

The analysis's findings demonstrated that the proximate composition of the fish balls was significantly impacted by the changes in the type of components and storage duration during 21 days. All fish ball samples showed significant differences in moisture, protein, lipid and ash content which were almost similar to the data reported by Kolekar and Pagarkar (2013). The results showed that the contents of catla fish balls in curry stored in refrigerated storage varied somewhat in terms of moisture (68.12 to 66.75%), protein (15.73 to 14.26%), lipid (8.75 to 11.05%) and ash (3.18 to 3.85%) at the start and finish of the storage period.

One of the criteria that determines a food product's quality is its moisture content, which also dictates how durable the food or food product is. A number of factors, such as the raw materials and processing method, influence the moisture level of fish balls, which in turn impacts the quality of the final product. The moisture content of fish balls was significantly impacted by the various additives and storage duration in the current study ($P<0.05$). The fish balls with pea flour in this trial had a somewhat lower moisture content than the others. Over the storage duration, the moisture content of all varieties of fish balls kept at 4°C increased. Another investigation revealed a similar pattern in moisture content where the moisture content of fish balls rose from 64% on the first week of storage to 75% on the twenty-first week (Ninan et al., 2008).

The addition of substances differed the protein level significantly from one another. The average protein content of fish balls without binding additives was 14.04±0.08%. When pea-flour, rice flour and wheat flour were added to fish balls, the protein content decreased to 12.51±0.03%, 11.86±0.06% and 11.42±0.05% respectively. This study showed slight decreased in protein content for all types of fish balls throughout the storage period of 21 days. The results obtained from the present study are more or less in agreement with Shikha et al., (2019). This may be due to the quantity of component used and the moisture content can also affect the protein content. Proteins' oxidative stability may be impacted by the reduction in protein concentration brought on by frozen storage, which could impact their ability to retain water (Alkuraieef et al., 2020). The amount of protein in meatballs can be influenced by enzyme activity and microbial activity. Protein denaturation will be accelerated by bacterial growth, resulting in a drop in protein levels.

The lipid content was significantly changed ($P<0.05$) with the addition of ingredients at various concentrations over the period of 21 days of storage. Fish balls with an addition of rice flour had a higher lipid content compared to others which had a 3.77±0.08% lipid content. Fish balls without binding ingredients had higher fat content than fish balls with the addition of binding ingredients which had a 2.39±0.04% fat content. During the storage period, on 21st day significant decrease ($p<0.05$) in lipid content was recorded which was reduced from 2.39±0.04% to 1.51±0.01% in fish ball without binding ingredients, 3.08±0.04% to 2.54±0.04% in fish ball with pea-flour, 3.69±0.04% to 3.29±0.02% in fish balls with wheat flour and, 3.77±0.08% to 3.13±0.02% in ball with rice flour. This may be due to unsaturated fatty acids in fats can undergo oxidative degradation even at low temperatures, leading to the breakdown of fat molecules. The oxidation process may produce peroxides, aldehydes, and other compounds that effectively reduce the measurable fat content.

The amount of inorganic material left over after combustion is known as the ash content. The amount of ash in a material is correlated with its mineral composition.

As the storage days went by, the average amount of ash in each kind of fish ball dropped. This could be because, particularly in high-moisture items like fish balls, the moisture released during storage may take soluble minerals away. Fish balls kept in a cold environment may lose some of their nutritional value due to dehydration, such as a reduction in ash content (Alkuraieef et al., 2020). However, Hutapea, (2010) noticed that there were no appreciable changes in the amount of ash when red tilapia fish balls were stored at room temperature in plastic packaging.

5.2.2.2. Determination of TVB-N Content of Prepared Fish Balls

Trimethylamine (TMA), dimethylamine (DMA), ammonia and other volatile basic nitrogenous chemicals linked to seafood spoiling are measured to determine Total Volatile Base Nitrogen (TVB-N). It is among the most used methods for determining how much seafood has spoiled.

The present investigation demonstrates how storage temperature affects product quality and freshness by seeing a notable rise in Total Volatile Base Nitrogen (TVB-N) levels in each type of fish balls throughout a 21 days storage period. The TVB-N values of all products were low at the beginning of the storage period. The TVB-N content in all types of fish balls showed increasing trend throughout the storage period, indicating microbial activity and protein breakdown, which were commonly associated with spoilage. Similar increases in TVB-N content were also reported by Kolekar, (2012), Dutta, (2009) and Ninan et al., (2008).

5.3. Total Plate Count of Prepared Fish Balls During Storage

Total plate counts in fishery products are a valuable technique for assessing shelf life and post-processing contamination (Duman and Ozpolat, 2012). The mean Total Plate Count (TPC) data for fish balls clearly shows the relationship between storage time and microbiological development, highlighting the influence of these factors on product quality and safety. In terms of microbial contents, the International Commission on Microbiological Specifications for Foods (ICMSF) and the Saudi Standards, Metrology and Quality Organization (SASO) define 1.0×10^7 and 5.0×10^5 CFU/g as the upper (rejectable) and lower (marginal) levels of acceptability, respectively, as acceptable and of good quality (Alkuraieef et al., 2020). As the storage duration increased proliferation of microbes was observed and TPC also increased significantly in each type of fish balls from 0 to 21 days of storage at 4^0C temperature. The fish ball with pea flour demonstrated the lowest TPC, ranging from 1.09×10^4 CFU/g to 2.84×10^5 CFU/g. These findings align with the findings of Kolekar and Pagarkar, (2013). The increase in TPC with the increase in storage duration was also reported by Mugale et al., (2015).

Therefore, this study indicates that the balls possess better acceptability and can be consumed up to 7 days of storage period for fish ball made with pea flour, wheat flour and rice flour. This study roughly corresponds to Singh et al., (2023)'s investigation into the sensory qualities and proximate composition of ready-to-eat fish balls made from Rohu (*Labeo Rohita*) mince and kept at refrigeration temperature where the fish balls exhibit superior acceptability up to the fourteenth day at 4±1°C in aerobic packaging.

CHAPTER 6

CONCLUSIONS

CHAPTER 6: CONCLUSIONS

This study examined how the quality and shelf life of fish balls made with different ingredients changed throughout a 21 days storage period at 4°C. It may be concluded that the formulation of fish balls with pea flour were more acceptable to the panelists and the fish balls made with wheat flour and fish balls containing rice flour were better, but the fish balls prepared without binding ingredients may not be accepted. Based on the proximate composition, biochemical parameters, sensory attributes and microbiological analysis of fish balls that are ready-to-cook/fry and made from tigertooth croaker surimi and kept at 4°C, it is evident that the balls have higher acceptability and can be eaten for up to 7 days after being stored. This is especially true for fish balls that contain pea flour, wheat flour and rice flour. Overall, this study demonstrated that using various ingredients for preparing fish balls and storage condition has significant effect on the quality of fish balls. Moreover, good handling and hygiene practice were essential for ensuring the microbial safety and quality of fish balls. The conclusions obtained could potentially serve as a foundation for the industry of Jewish products. Utilizing tigertooth croaker for the creation of innovative fish ball products might be a lucrative alternative because they are inexpensive fish with limited acceptance for fresh consumption due to a number of restricting issues. The nutritional components and functional characteristics of fish balls made from these fish may be thoroughly examined in future research.

CHAPTER 7

FUTURE RECOMMENDATIONS

CHAPTER 7: FUTURE RECOMMENDATIONS

Improving the quality and shelf life of fish balls involves optimizing production, storage, and packaging processes. In order to ensure the quality and extend the shelf life of fish balls, it is essential to implement proper handling, storage, and packaging techniques. Here are some recommendations:

- High-quality, fresh fish should use as the primary ingredient to ensure better texture, flavor and longer shelf life.
- Additives and other ingredients should be safe and contamination free to reduce microbial load
- Strict hygiene standards should maintain in processing areas to prevent contamination (e.g. use clean, sanitized equipment and follow Good Manufacturing Practices (GMP).
- Thiobarbituric acid (TBA) levels and peroxide values should monitor to assess spoilage
- Enzymatic activity (lipase, protease) should evaluate as potential spoilage indicators
- Research should conduct on the effects of freezing, chilled storage, and different thawing methods on texture and taste for optimizing storage condition

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Appendix A

Product Evaluation Form

Name:	Product name: Fish Ball
Panelist no:	Date:

Instructions: You are kindly requested to taste the given samples, then place an "X" mark on the point in the scale which best describes your feelings

Sample code	Rating scale	Color	Flavor	Texture	Overall acceptability
	8 to 10	Contents appropriately colored (bright brown)	Contents have no abnormal odor and have a very good characteristic flavor and seasoning	Hard, highly elastic	Excellent
	6 to 7	Contents generally acceptable colored (brown/white)	Contents have no abnormal odor, flavor and seasoning, satisfactory flavor	Moderately hard and elastic	Very good
	4 to 5	Contents moderately colored (grayish)	Contents have slightly raw or scorched odor or flavor; seasoning seems to be somewhat inadequate	Slightly soft and elastic	Good
	1 to 3	Contents considerably discolored (dark gray)	Contents have strong abnormal odor and a poor flavor	Soft and broken, no elasticity	Poor

Fish ball with pea flour					
Fish ball with wheat flour					
Fish ball with rice flour					
Fish ball without ingredients					

Remark/ Observation

....
....
....

Appendix B

Table: Organoleptic Quality Assessment of Fish Balls Under Chilled Storage Condition

Sample	Characteristics	Storage days			
		0	7	14	21
Fish ball with pea flour	Color	Bright brown	Bright brown	Brown	Brown
	Flavor	A very good characteristics flavor	Good characteristics flavor	Poor flavor	Poor flavor
	Texture	Hard and highly elastic	Hard and elastic	Moderately hard and elastic	Moderately hard and elastic
	Overall acceptability	Excellent	Very good	Good	Poor
Fish ball with wheat flour	Color	Bright brown	Bright brown	Brown	Brown
	Flavor	A very good characteristics flavor	Good characteristics flavor	Poor flavor	Poor flavor
	Texture	Hard and highly elastic	Hard and elastic	Moderately hard and elastic	Moderately hard and elastic
	Overall acceptability	Excellent	Very good	Good	Poor
Fish ball with rice flour	Color	Bright brown	Bright brown	Brown	Brown
	Flavor	A very good characteristics flavor	Good characteristics flavor	Poor flavor	Poor flavor
	Texture	Hard and highly elastic	Hard and elastic	Moderately hard and elastic	Moderately hard and elastic
	Overall acceptability	Excellent	Very good	Good	Poor
Fish ball without ingredients	Color	Brown	Brown	Grayish	Grayish
	Flavor	Slightly raw or scorched odor or flavor	Slightly raw or scorched odor or flavor	Poor flavor	Poor flavor
	Texture	Slightly soft and elastic	Slightly soft and elastic	Soft and broken	Soft and broken

	Overall acceptability	Good	Good	Poor	Poor

Photo gallery

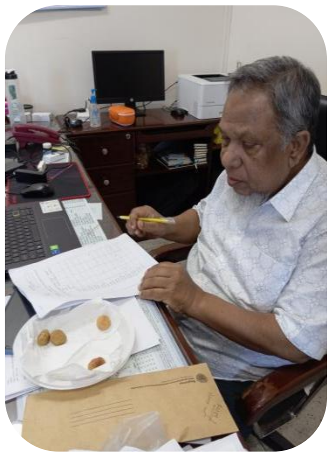
Plate 1: Fish Ball Preparation

Mixing

Removing water

Packaging

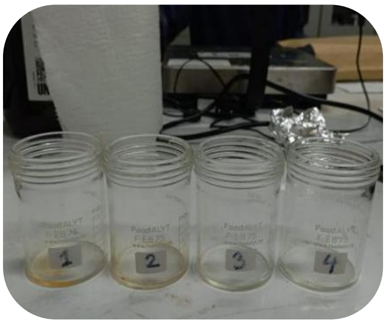
Frying



Panel test

Plate 2: Biochemical Analysis Work

Weighing sample **Moisture determination**



Lipid determination **Titration**

Homogenization **Filtration**

Plate 3: Microbiological Work

Instruments

Pouring agar

Sample preparation

Bacterial colony

Bacterial colony counting

Brief Biography of the Author

Suchita Chakma, Daughter of Laxmi Moni and Kanchan Mala Chakma from Rangamati Sadar Upazila of Bangladesh. Now she is the postgraduate student of Department of Fishing and Post-Harvest Technology, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh. She passed her graduation from the same university in 2021. She passed her Higher Secondary Certificate Examination in 2017 from Rangamati Govt. College, Rangamati and Secondary School Certificate Examination in 2015 from Rangamati Govt. Girls' High School, Rangamati. She has a great interest on doing different scientific research.