

**PROXIMATE COMPOSITION AND ISOLATION OF COLLAGEN
FROM PELAGIC AND DEMERSAL FISH (*SPHYRAENA OBTUSATA*
AND PSETTODES ERUMEI) IN COASTAL REGION OF CHENNAI.**

PROJECT WORK

Submitted in partial fulfilment of the requirements for the award of a Bachelor of
Science degree in Biochemistry

By

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SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

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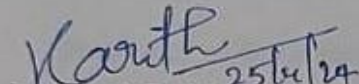
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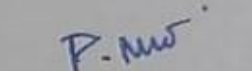
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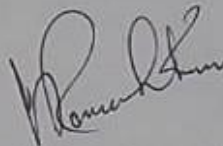
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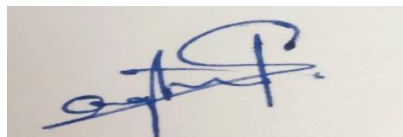

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DECLARATION

RAGATHI SARAVANAN (41070006) hereby declare that the Project Report entitled **“PROXIMATE COMPOSITION AND ISOLATION OF COLLAGEN FROM PELAGIC AND DEMERSAL FISH (*SPHYRAENA OBTUSATA* AND *PSETTODES ERUMEI*) IN COASTAL REGION OF CHENNAI.** “is my original work conducted under the supervision of **Dr. P. Marimuthu, Assistant professor (research) , Centre for Ocean Research, Col. Dr. Jeppiaar Research Park ,Sathyabama Institute of Science and Technology** is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Biochemistry.

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ABSTRACT

Fish collagen has gained significant attention due to its potential applications in various industries, including food, pharmaceuticals, and cosmetics. This study focuses on investigating the proximate composition and isolation of collagen from two distinct fish species, *Sphyraena obtusata* (pelagic) and *Psettodes erumei* (demersal), harvested from the coastal waters of Chennai, India. The proximate composition analysis involves determining the moisture content, protein content, lipid content, and ash content of the fish samples using standard analytical methods. Furthermore, collagen isolation includes acid-solubilization will be employed to extract collagen from the fish skin. Further the molecular weight is determined . A comparative analysis between the collagen extracted from the *Sphyraena obtusata* and *Psettodes erumei* will be conducted to evaluate their suitability for various applications. The findings of this research will provide valuable insights into the proximate composition and collagen characteristics of pelagic and demersal fish species from the coastal region of Chennai, contributing to the utilization of fishery by-products for value-added products and sustainable development.

KEYWORDS: *Sphyraena obtusata*, *Psettodes erumei*, proximate, collagen isolation, pelagic and demersal

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LIST OF ABBREVIATIONS

- % -Percentage
- Cm - centimeter
- OD- optical density
- PBS- phosphate Buffer Saline
- μL - Microliter
- UV-Ultra Violet spectrometer
- SDS-PAGE – Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

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CHAPTER 1

INTRODUCTION

The marine environment, consisting of 70% of the Earth's surface, represents one of the last frontiers of scientific exploration and discovery to mankind. The study of marine science holds a variety of disciplines, ranging from oceanography and marine biology to marine geology and coastal engineering. Researchers in these fields strive to unravel the mysteries of the sea, seeking to understand the complex processes that govern marine environments and the organisms that inhabit them. This gained knowledge empowers us to conduct future research that not only advances mankind but also addresses pressing global issues, such as food scarcity, medicinal innovation, etc.,

The dynamics of global fish production are heavily influenced by China, which contributes approximately 32 percent of the world's total production. Other significant contributors include Japan, India, the United States, the Russian Federation, and Indonesia.

Over the years, there has been a notable increase in per capita fish consumption worldwide, rising from approximately 9 kg per annum in the early 1960s to about 16 kg in 1997. However, in India, there hasn't been a significant change in per capita consumption due to the substantial increase in population. According to the Ninth Plan document, India's per capita consumption was estimated at 9 kg per annum, assuming that 56 percent of the Indian population consumes fish. As part of the tenth plan, a target is to increase per capita consumption to 11 kg per annum.

India boasts extensive inland water resources, including rivers and canals spanning 0.2 million kilometers, reservoirs covering 3.1 million hectares, and tanks and ponds totaling 2.2 million hectares, providing ample opportunities for fish production. The vast network of Indian rivers, represents a crucial asset for inland fisheries. However, unsustainable fishing practices have contributed to the decline in riverine fish production. Fish farming in inland areas has grown the most and become the most

important part of the fishing industry. Despite their significance, the multifaceted use of inland waters has often relegated fisheries to a lower priority in many states, as the concept may be novel to fishing communities and necessitates high maintenance. Consequently, the neglect has led to habitat degradation in most inland water resources, resulting in diminished fish yields and a corresponding decrease in rural employment opportunities.

Likewise, coastal communities rely heavily on marine fisheries as a primary means of sustenance. Although inshore fishing has reached its capacity, there's vast potential waiting to be tapped in deep-sea fisheries, mariculture, and enhancing value post-harvest. Unlike river species, marine species often hold greater commercial value, known as capture fisheries that sustain livelihoods and import-export worldwide trade in economics which includes India's blue revolution scheme with an average rate of 9% a year production and accessibility to a variety of seafood. Moreover, the impact of environmental factors, including natural disaster fluctuations and weather patterns, affects the feeding habits of marine fishes, influencing the growth rate, breeding Platform, and distribution patterns of marine populations. *Mohan Dey Madan et al., (2005).*

1.2 DEMERSAL AND PELAGIC FISH

Demersal fish inhabit the seabed, with flattened bodies suited for bottom-dwelling. Examples include cod (*Gadus spp.*) and flounder (Pleuronectidae family). Pelagic fish, found in open water, have streamlined bodies for efficient swimming. Tuna (*Thunnus spp.*) and mackerel (*Scomber spp.*) are common pelagic species. Understanding these distinctions is crucial for effective fisheries management and conservation efforts *Watson et al.,(2012)*. The study is focused on two prominent marine fish species, *Sphyraena obtusata* (Barracuda) and *Psettodes erumei* (Indian Halibut) based on their feeding habits

Table1.2 Data comparison between pelagic and demersal finfish (Watson et al.,2012)

Category	Landings (million tonnes)	Percentage
Pelagic finfish	1.43	54.8
Demersal finfish	0.65	24.9

The study is focused on two prominent marine fish species, *Sphyraena obtusata* (Barracuda) and *Psettodes erumei* (Indian Halibut).

1.2.1 *Sphyraena obtusata* (Barracuda)

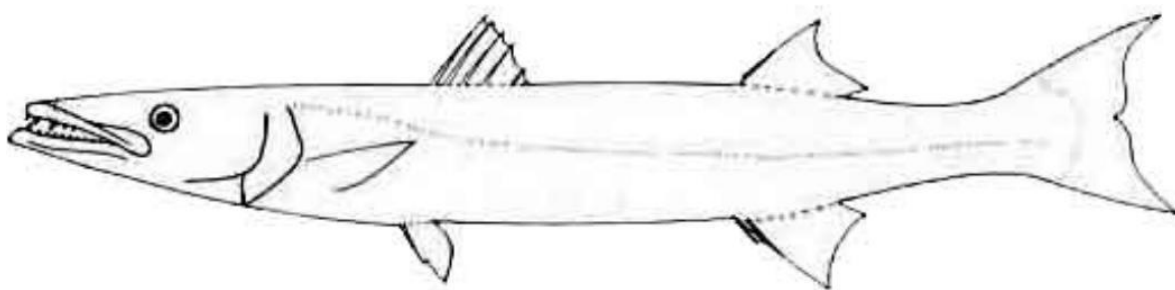


Fig:1.2.1 *Sphyraena obtusata* (Barracuda)

The Barracuda, scientifically known as *Sphyraena obtusata*, is an imposing figure in the world's tropical and subtropical oceans. *Sphyraena obtusata* are found in marine and brackish waters, and inhabit subtropical climates, ranging between 42°N - 35°S and 180°W - 180°E. They are typically found at depths ranging from 3 to 30 meters, even though they can occur from 0 to 100 meters. *Sphyraena* are renowned for their prowess as hunters and their distinct physical characteristics.

Sphyraena obtusata mostly feed primarily on fishes, cephalopods, and occasionally shrimps. The *Sphyraena obtusata* is a formidable piscivore, as it utilizes its acute visual and olfactory senses to locate its prey. They have an elongated, slightly compressed body, accentuated by a large head with a long pointed snout. Their mouth, equipped

with weak canine teeth on both the lower jaw and the palate, facilitates efficient predation. Additionally, they possess two short and widely separated dorsal fins, a dirty yellow-colored anal fin, a second dorsal fin, and a caudal fin. Despite their predatory nature, *Sphyraena obtusata* are typically grey/blue with a whitish tinge on their upper body and a silvery ash line along the sides, aiding in their camouflage as they stalk their prey.

They are widely preferred by the south Indians as they provide the major source of nutrition. Because of this reason they are a targeted species for fishing, caught by handline, set nets, and various other fishing gear. However, despite their fearsome reputation, interactions with humans are rare. *Sphyraena obtusata* rarely attack humans, usually employing one quick, fierce strike, which, though serious, is seldom fatal.

1.2.2 *Psettodes erumei* (Halibut)

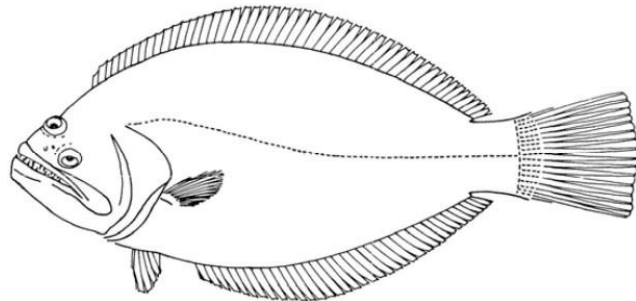


Fig: 1.2.2 *Psettodes erumei* (Halibut)

Psettodes erumei commonly known as the Indian Halibut inhabits the vast expanse of the Indo-West Pacific region, spanning from the Red Sea and East Africa all the way to Japan and Australia.

Their flat and elongated body shape, laterally compressed to camouflage on the ocean floor, distinguishes them among flatfish species. *Psettodes erumei* asymmetrically positioned eyes to aid in camouflaging and maintaining a clear view of their surroundings while lying in wait for prey. Their mottled coloration further enhances camouflage against sandy or rocky seabeds. *Psettodes erumei* possess a dorsal fin

running along their back, an anal fin underneath, and pectoral fins for maneuvering. Large mouths equipped with sharp teeth facilitate capturing and holding onto prey. Covered in small, overlapping scales, *Psettodes erumei* skin is smooth and slippery, aiding in streamlined movement through water. Highly valued for its culinary appeal and known for its nutritional value that is excellent source of lean protein, vitamins B12 and B6, niacin, phosphorus, selenium, and omega-3 fatty acids.

Psettodes erumei offers a mild, sweet flavor and firm texture, making it a preferred seafood choice over *Sphyræna obtusata*. Despite their differences, when it comes to nutritional and commercial value, a great population prefers them. This was taken into account to carry out a brief study on the respective species. The study is subdivided into two parts; proximate analysis in the muscle sample and collagen extraction from the skin.

1.3 PROXIMATE ANALYSIS

The increasing global population and their growing preference for nutritional dietary choices have led to an immediate preference for seafood consumption. The consumption of seafood compared to white meat, as a day-to-day meal has increased in today's healthy lifestyle. Even though beef and egg provide high protein content yet, fish is highly consumed as it provides amino acid that helps in the synthesis of protein during growth and other cellular activities Beveridge, J. M. R,(2011). Fish is also rich in omega-3 fatty acids which may help to reduce symptoms of rheumatoid arthritis and may even help to improve brain and eye health Yetty Natalia *et al.*, (2003). Regular consumption of fish can reduce the risk of cancer, such as breast, colon, and prostate, and lower the risk of Dementia, and Alzheimer's disease, Many prevent cardiovascular diseases E. Balk *et al.*, (2004). The main components of fish are 66%–81% water(moisture), 15%–20% protein, 1.2%–1.5% mineral, 0.5%–20% fat,0%–0.5% carbohydrate; 96%–98% of the body composition of fish is constituted by moisture, protein, fat, and ash content. To determine these biological factors, the following methodology is known as “proximate analysis” Bimal Prasanna Mohanty, (2015).

1.3.1 PROTEIN

In fish, the protein content of muscle tissue typically ranges from 16% to 20%, varying based on muscle type. Dark muscles generally exhibit lower levels of both moisture and protein compared to light muscles. Connective tissue proteins, also known as stroma proteins, make up approximately 3% of the protein content in teleosts and around 10% in elasmobranchs, contrasting with the 17% found in mammals. This lower collagen content contributes to the soft texture of fish meat. Collagen, the primary connective tissue protein in fish, closely resembles collagen from mammals. The amount of collagen in different fish species' body tissues varies, possibly linked to their swimming behavior. In fish, pelagic species generally exhibit higher levels of sarcoplasmic protein, whereas demersal species tend to have lower concentrations.

Per unit weight, fish provides a substantial amount of protein, typically ranging from 18% to 20%. It also offers all eight essential amino acids, including sulfur-containing lysine, methionine, and cystine. Additionally, amino acids such as aspartic acid, glycine, and glutamic acid contribute to the wound-healing process. Chyun and Griminger, (1984).

1.3.2 LIPID

Lipids encompass a diverse range of compounds extracted using low-polarity solvents. Remarkably, each gram of fat offers over twice the energy of carbohydrates and proteins, providing 9 kilocalories per gram. There exist five main categories of fat: saturated, monounsaturated, polyunsaturated, trans-fatty acids, and omega-3 fatty acids. Within fish muscle, lipids represent the third major component by quantity.

Fish can contain fat levels ranging from as low as 0.5% to as high as 18-20%. Importantly, the fat in fish comprises a significant proportion of omega-3 and other unsaturated fatty acids, which are notably healthier compared to the saturated fat found in red meat. Yetty Natalia *et al.*, (2003).

1.3.3 CARBOHYDRATE

in fish, carbohydrates play a role in providing energy for metabolic processes. While fish generally have lower carbohydrate content compared to other macronutrients like protein and fat, they still serve as a source of energy. The specific carbohydrate content and composition in fish can vary depending on factors such as species, diet, and environmental conditions. Additionally, the presence of carbohydrates in fish diets can influence growth, metabolism, and overall health

1.3.4 WATER

The water or moisture content in fish is a critical aspect of its composition, affecting its quality, texture, and nutritional value. Generally, fish have high moisture content, typically ranging from 60% to 80% of their total weight. However, this percentage can vary depending on factors such as species, age, diet, habitat, and handling practices. It contributes to the succulence and tenderness of the fish meat, making it more palatable and enjoyable to consume.

In the fisheries and seafood industry, the conscientious task of gauging the intrinsic value (energy value) of fish and strategizing the optimal methods for their industrial and commercial processing hinges upon a comprehensive understanding of their proximate composition. It acts as a good indicator to understand the medicinal value of fish to prevent allergies and separation of fish based on edible and non-edible. Due to the tremendous change in the climate condition, season, and industrial growth, there could be wide differences in the biochemical constituents of the fish by doing proximate analysis we can develop the area where the fish growth is maintained Alistair J. Hobday and Karen Evans (2013) .Hence it becomes essential to document the proximate composition of the fishes periodically in a region. Proximate analysis is done widely across the globe to study the composition of marine animals in different regions and under different climatic conditions.

1.4 COLLAGEN

Marine resources, particularly fish waste, offer a significant and cost-effective source of collagen for various medical applications. Fish collagen, derived from fish species' skin, scales, and bones, has emerged as a promising alternative to mammalian collagen because of its unique composition and numerous health benefits Daniela Coppola *et al* ., (2021). In addition to their great disadvantage, which is a high rate of contaminated heavy metal found in the product, Fish collagen extracted from the by-products exhibits superior properties, including excellent biocompatibility and fewer religious or ethical concerns compared to collagen from other sources like porcine or bovine.

1.4.1 TYPES AND STRUCTURES OF COLLAGEN

Collagen possesses a distinctive amino acid composition, with glycine (Gly), proline (Pro), hydroxyproline (Hyp), and hydroxylysine (Hyl) being predominant. Glycine comprises one-third of the sequence. These amino acids are organized in a repetitive tripeptide sequence, Gly-X-Y, where X typically represents hydroxyproline or hydroxylysine, although it can be any amino acid. Collagen is classified into various types ranging from 1 to 24 Type. In fish, the predominant type of collagen found is Type I collagen, comprising the majority of the collagen present

1.4.2 COLLAGEN FORMATION

Collagen formation involves various cell types, including mesenchymal derivatives like fibroblasts, odontoblasts, osteoblasts, cementoblasts, and chondroblasts. Initially synthesized as procollagen, messenger RNA guides the assembly of amino acids into polypeptide chains in the rough endoplasmic reticulum (RER). These chains undergo post-translational modifications, including hydroxylation of lysine and proline, facilitated by vitamin C-dependent enzymes. Further glycosylation occurs in the RER, followed by triple helix assembly and alignment through disulfide bonding. The mature collagen is then transported to the Golgi complex for final glycosylation before being packaged into secretory granules and released via exocytosis. This process takes around 35 to 60 minutes.

Following the formation of procollagen, enzymatic cleavage occurs at both ends facilitated by enzymes like C-procollagen peptidase and N-procollagen peptidase. Subsequently, lysine oxidation takes place in the presence of copper ions (Cu^{2+}). In the final step, aldol condensation occurs, leading to the production of tropocollagen, which serves as the robust and stable form of collagen, recognized as the functional and structural unit. Tropocollagen acts as the precursor from which various collagen structures arise, including long fibrils (such as types 1, 2, and 3) and mesh networks (like types 9 and 11) are synthesized Sharma *et al.*, (2009).

Research has explored its potential in biomedical applications such as wound healing, tissue engineering, and drug delivery with the help of collagen. However, further understanding of fish collagen's physicochemical properties is needed to address its disadvantages. Biomaterial-based fish collagen is being explored to overcome drawbacks and develop composites with ideal properties. Despite challenges, fish collagen shows promise for medical product development thus continued research efforts are essential to fully explore the potential of fish collagen across a range of biomedical areas, ultimately contributing to advancements in healthcare and improved patient outcomes Hafez Jafari *et al.* , (2020).

CHAPTER 2

REVIEW OF LITERATURE

2.1 PROXIMATE ANALYSIS

The literature review section delves into existing research on proximate analysis carried throughout the marine technologist both national and international sector, with respect to above variations. *Sardinella longiceps* locally known as Oil sardine (11% fat) is well known for its fat content in India , but a study suggests that among the fish species surveyed along the East Coast of India, *Leiognathus dussumieri* exhibits the highest fat content, exceeding 14% fat Palani,(2014). Lipid content can vary from fish to fish from lean fish to fatty fish (5%-20% fat) yet fishes that come under low-fat distributors found in Arabian gulf region constitute below 3% of the total nutritional value M. Zamil El-Faer,(1991).

Fish caught from the Black Sea, including species like *Sarda sarda* (Bonita), *Engraulis encrasicolus* (Anchovy), and *Merlangius merlangus* (Whiting), are extensively consumed by the populations of Russia and Ukraine. A study focusing on fish originating from the Black Sea revealed that each of these species contains approximately 18% lipid content. As a result, they come highly recommended by dietitians and nutritionists for inclusion in diets Saadettin Guner,(1988).

P. Barua *et al.*,(2012) have analyzed proximate composition and macro and micronutrients in string ray fish such as *Dasyatis americana* (Southern stingray), *Dasyatis pastinaca* (Common stingray), *Dasyatis zugei* (Zuge's stingray) from Bangladesh coastal area .Similarly comparative proximate analysis was carried out in catfish where only moisture and protein content was surveyed showed that *Mystus vittatus* (Striped Dwarf Catfish) had notably higher moisture content than other varieties. This elevated moisture content correlated with increased deterioration levels during prolonged storage, attributed to heightened microorganism activity Gita Bezbaruah,(2020).

Whereas in the Gulf of Mannar proximate analysis and metal distribution was carried out in by-catch Pufferfish were high metal activity (cadmium, copper, lead and zinc) was found in , Liver, Kidney, Gill, Intestine sample compared to other edible fish in Tuticorin K. Karunanidhi *et al*, 2017 .

Likewise, a Proximate analysis was carried out based on the sexes of fish in the Gulf of Lake Tana, Ethiopia. Results indicated variations in protein content between male and female specimens of *Labeo intermedius* (Intermediate labeo), with values of 14.5 ± 0.58 for males and 16.0 ± 0.58 for females. Similarly, in *Clarias gariepinus* (African catfish), fat content varied between males and females, with respective values of 0.8 ± 0.05 and 2.3 ± 0.05 . (Hirut Geremew, 2020). k sujatha *et al*, 2013 studied total protein and lipid content in species such as *Rastrelliger kanagurta* (Indian mackerel) , *Mugil cephalus* (Striped mullet), *Nemipterus japonicus* (Japanese threadfin bream) *Spyraena obtusata* (Indian barracuda) captured from fish center Kasimodu , India.

Proximate composition was found in various section of Flesh in Atlantic Halibut (*Hippoglossus hippoglossus*) in Canada A. Mannan *et al*, 2023. A publication from the 1970s presented data on 155 species, encompassing finfish, mollusks, and crustaceans. The data included nutritional information such as protein, fat, moisture, ash, carbohydrate, energy value, and cholesterol content, all derived from the raw (fresh or frozen) flesh parts of these species Virginia D. Sidwell *et al*, 1973 .

Proximate analysis along with amino acid and fatty acid content was conducted on flat fish such as *Barbodes guibei*: Tawes , *Pterophyllum scalare*: Angelfish etc in cuddalore region of tamil nadu Ramasamy, Manikandan, et al, (2023). Similarly Amino acid content was determined in Atlantic halibut (*Hippoglossus hippoglossus*), yellowtail flounder (*Pleuronectes ferruginea*) and Japanese flounder (*Paralichthys olivaceus*) Jeong Dae Kim and Santosh Lall, (2000). Proximate analysis was carried out in four tropical fishes in Nigeria Victoria O. E. Akpambang, (2015).

The protein content significantly rose in the sequence of frying > grilling > boiling among the three fish species (*Polydactylus quadrifilis*: Threadfin scad, *Chrysichthys nigrodigitatus*: Blackspotted catfish, *Cynoglossus senegalensis*: Senegalese tongue

sole) subjected to proximate analysis and metal detection based on processing methods. Frying induced the greatest moisture loss, resulting in the highest protein levels, thus give us a conclusion that elevated protein levels in fried and microwave-cooked fish compared to raw varieties F. I. Bassey *et al.*, (2014).

Ananthan, G *et al.*, (2012) have studied seasonal changes in proximate composition in *ascidians* sea squirts . Similarly Khawaja and Jafri (1968b and 1970) studied on seasonal variations of calcium and phosphorus in the juveniles of *Ophicephalus punctatus* and *Cirrhinus mrigala*. Seasonal variations in chemical and sensory characteristics was carried out in the wild and farmed Atlantic halibut (*Hippoglossus hippoglossus*) by Olsson, Gunn Berit, *et al.*, (2003)

This part of the review of the literature reveals that factors such as geographical location, seasons, and management practices significantly impact the body composition and nutritional value of fish. However, detailed studies on the nutritional status of fish concerning species, seasons, habitats, and production systems, particularly regarding Barracuda and Halibut found in the coastal area of Kovalam region in Southeast India, are lacking. Given this gap, there is an urgent need to conduct research on the proximate composition, including moisture, crude protein, crude fat, and carbohydrates, of selected edible fish species from both pelagic and demersal categories, such as *Sphyrna obtusata* and *Psettodes erumei* , originating from the state of Tamil Nadu.

2.2 FISH COLLAGEN

The term "collagen" derives from Greek roots, with "cola" meaning glue and "genno" signifying birth. Consequently, collagen acts as a binding agent, facilitating the formation of tissues and organs within the body. Comprising approximately one-third of the total protein content in humans, it constitutes three-quarters of the dry weight of skin and stands as the predominant constituent of the extracellular matrix

Currently, research has identified a minimum of 29 distinct variations of collagen (Type-I-XXIX) Veeruraj *et al.*, 2015; Le *et al.*, 2014. Collagen is composed of three polypeptide

chains intricately intertwined to create a triple helix structure, primarily stabilized through intra- and inter-chain hydrogen bonding (Asghar and Henrickson, 1982). The triple helix pattern consists of a sequence triplet, Gly-X-Y, where Gly represents the amino acid glycine, while X and Y typically denote proline and hydroxyproline amino acids (Shoulders and Raines, 2010). This particular arrangement of collagen's structure can be disrupted through denaturation or melting processes. Moreover, various types of collagen exhibit distinct distributions across animal tissues.

Basically, it was mammals that extracted collagen (bovine and pig) which was majorly utilized, yet hazardous outbreaks such as bovine spongiform encephalopathy (BSE), transmissible spongiform encephalopathy (TSE), and foot and mouth disease (FMD) have constrained their utilization (Clare R Trevitt and Pramila N Singh, 2003).

In response, researchers have identified an alternative collagen source characterized by minimal immunogenicity, biocompatibility, and reduced risk of transmissible diseases. Fish waste, including scales, skin, and bones, is rich in protein, serving as a substantial alternative to mammalian collagen (Benedetto, Cristiano, *et al.*, 2014). In collagen extraction, two main methods are utilized: acid-based soluble collagen and pepsin-soluble collagen, these are extracted such as from tilapia fish skin (Sun, Leilei, *et al.*, 2017) and silver catfish (*Pangasius sp.*) skin (Hukmi, N. M. M., 2017).

Collagen extracted from Sponge (*Dendrilla Membranosa*) yielded a comparatively lower amount of collagen compared to that obtained from the skin of bigeye snapper (*Priacanthus tayenus* and *Priacanthus macracanthus*). (George *et al.*, 2018, S Benjakul *et al.*, 2009). Additionally, collagen has been successfully extracted from various parts of the bighead carp (*Hypophthalmichthys nobilis*), including fins, scales, skins, bones, and swim bladders (Liu, Dasong, *et al.*, 2012).

Collagen has been isolated and characterized from various marine sources such as skin of Bamboo shark (*Chiloscyllium punctatum*) (Kittiphattanabawon, Phanat, *et al.*, (2009), outer skin of Diamondback Squid (*Thysanoteuthis rhombus*) (Takeshi Nagai (2004), swim bladders of yellowfin tuna (*Thunnus albacares*) (Kaewdang Onouma, *et al.*, 2014) muscle of marine prawn (*Penaeus indicus*) (Sivakumar P *et al.*, (1996).

In his study, Jakub Hadzik along with his co-professor suggested the potential utility of fish collagen derived from silver carp (*Hypophthalmichthys molitrix*) skin is used as a biomaterial for bone healing and regeneration where there was no allergic or infection discovered in 15 treated rats. Jakub Hadzik, *et al.*, (2016). Similarly, collagen Type 1 was extracted for application in tissue bioengineering Ibrahim N Amirrah *et al.*, (2002). The application of collagen extracted from marine species extends beyond bone regeneration and tissue bioengineering. Additionally, researchers are exploring the utilization of newly discovered jellyfish (*Scyphozoa*) collagen for the development of microparticles intended for protein delivery Calejo, M. Teresa, *et al.*, (2012) . Another vital bio-application of collagen is wound healing, wherein collagen extracted from Tilapia (*Oreochromis niloticus*) and Grey mullet (*Mugil cephalus*) is utilized for repair purposes Manal Shalaby *et al.*, (2019) .

This section of the literature review highlights on fish collagen, which has gained prominence as an alternative source compared to traditional collagen derived from cows and pigs. While collagen from these sources has been utilized historically, evolving religious beliefs and concerns about health risks have led people to seek alternatives. Fish collagen has emerged as a viable option in response to these shifting preferences and considerations. Consequently, there has been a surge in interest in fish collagen, derived from underutilized fish by-products, which present a valuable resource. While fish collagen shows promise in various applications such as tissue wound healing, regeneration, and bone repair, there is a noticeable lack of comprehensive studies, particularly focusing on collagen extraction from species like Barracuda(*Sphyraena obtusata*) and Halibut (*Psettodes erumei*) in the Kovalam region of Southeast India. This research gap emphasizes the urgent need for in-depth investigation into the extraction of acid-soluble collagen from selected edible fish species, including *Sphyraena obtusata* and *Psettodes erumei* , originating from Tamil Nadu. Such endeavors can greatly enhance our understanding of fish collagen's potential applications and contribute significantly to the field of biochemistry.

CHAPTER 3

AIM AND OBJECTIVE

AIM:

To Investigate the proximate composition in muscle tissue of *Sphyraena obtusata* (pelagic) and *Psettodes erumei* (demersal) and Isolate collagen from the skin aforementioned fish species from coastal waters of Chennai, India.

OBJECTIVE:

To determine moisture, protein, lipid, and ash content of the fish samples

To isolate collagen from fish skin.

To Characterize the extracted collagen for physicochemical properties such as molecular weight distribution.

To perform a comparative analysis between collagen extracted from *Sphyraena obtusata* and *Psettodes erumei* to assess differences in composition and characteristics .

To Evaluate the potential applications of the isolated collagen in various industries such as food, pharmaceuticals, and cosmetics.

To Contribute to sustainable development by utilizing fishery by-products for value-added products and processes.

CHAPTER 4

MATERIALS AND METHODS

4.1 SAMPLE COLLECTION AND DISSECTION

Sphyraena obtusata (Barracuda) and *Psettodes erumei* (Indian halibut), were preserved in an insulated ice box during transportation and stored in a cold storage room until further processing. Upon removal from storage, they were immediately washed in normal water to eliminate any freezing effects. Non-edible parts such as the head, fins, scales, and viscera were removed, and the skin and flesh were carefully separated using a dissection kit. The skin and flesh were then placed into labeled plastic containers for further analysis. (Fig 4.1.1 and Fig 4.1.2)

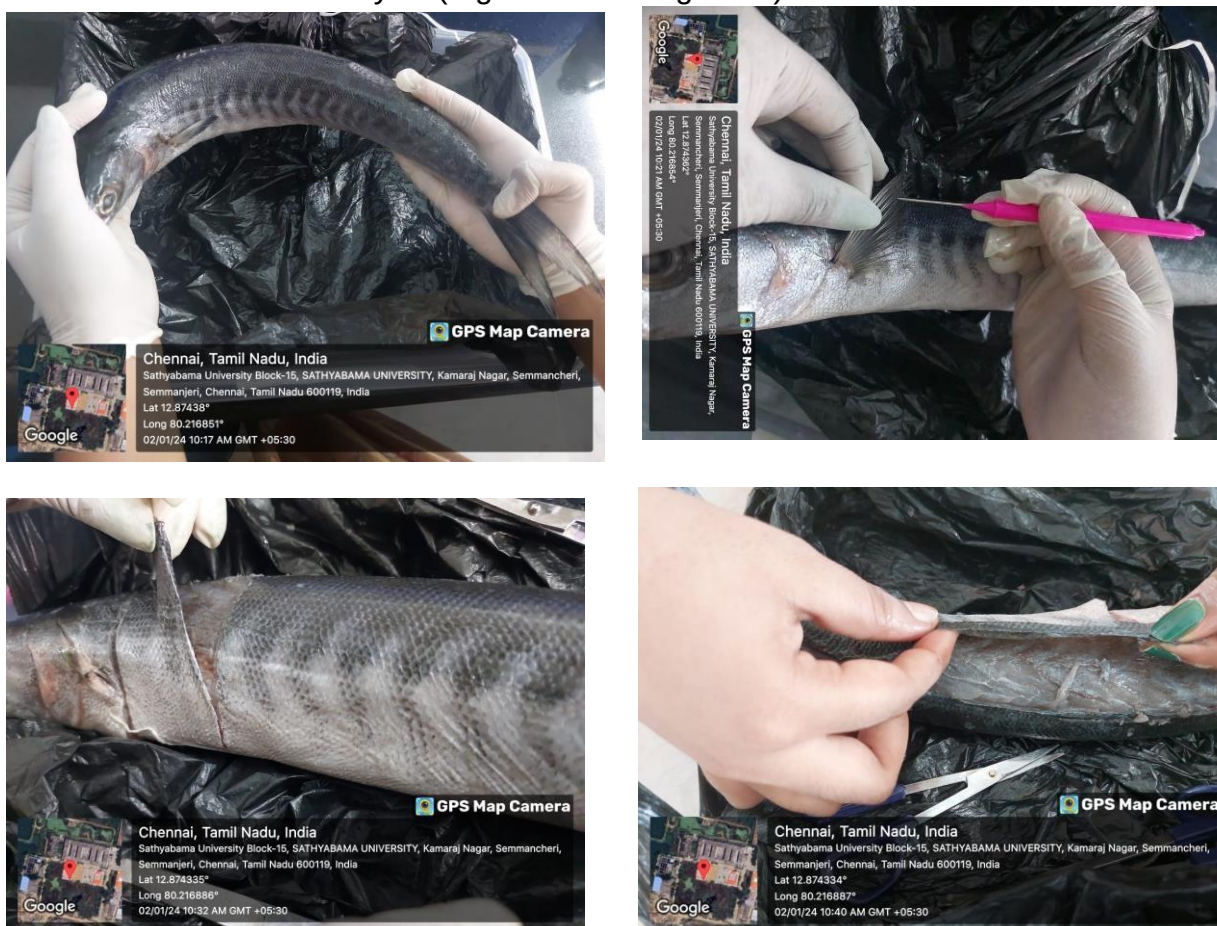


Fig 4.1.1 Dissection of *Sphyraena obtusata* (Barracuda)

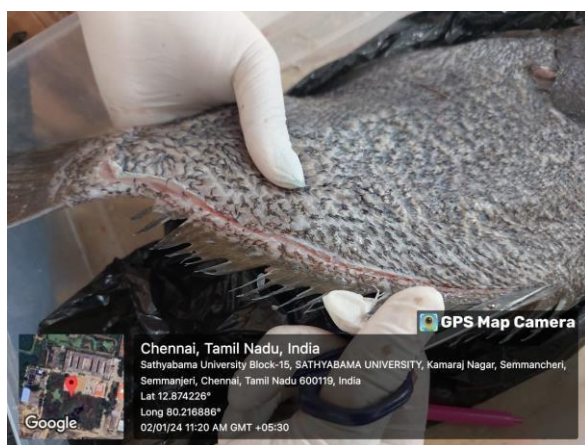
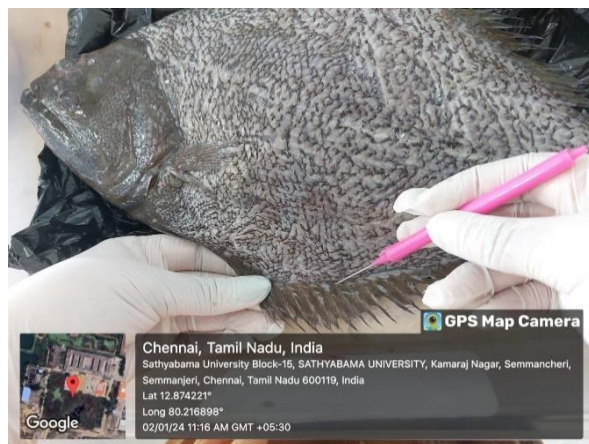


Fig 4.1.2 Dissection of *Psettodes erumei* (Indian halibut)

Notably, *Sphyræna obtusata* yielded a greater amount of flesh compared to *Psettodes erumei*. However, the skin of the *Psettodes erumei* proved challenging to peel due to its slimy and slippery nature, unlike the *Sphyræna obtusata*. To address this, the *Psettodes erumei* was thoroughly rinsed with cold water to remove excess muscle residue. Additionally, muscle samples were washed until completely free from any blood remnants.

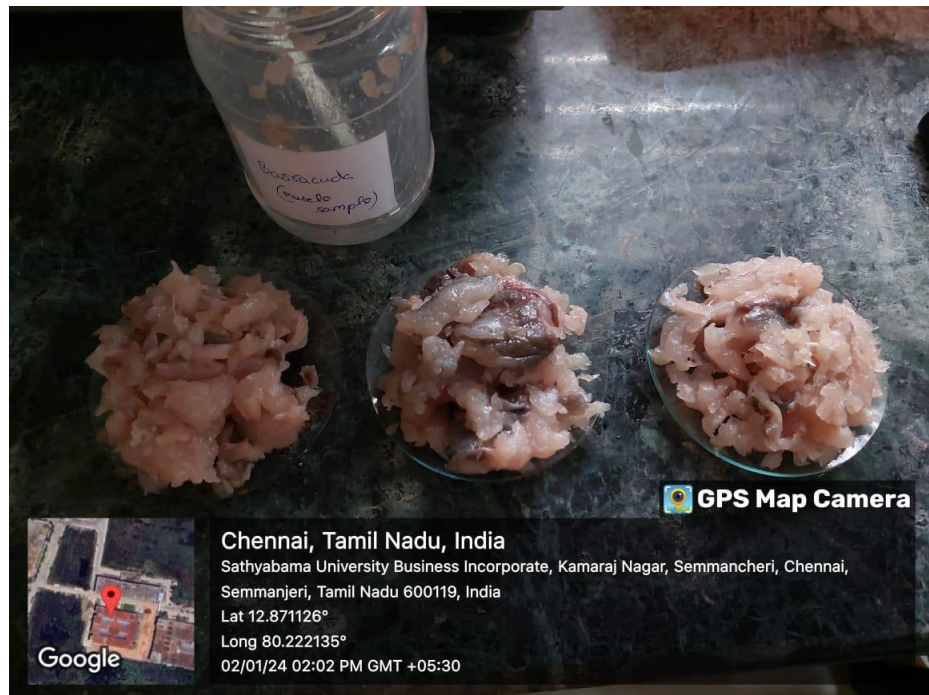
4.2 PROXIMATE ANALYSIS

Proximate analysis in fish involves assessing its nutritional composition, including moisture, protein, and carbohydrate content. This analysis helps determine the nutritional quality of fish, aiding in dietary recommendations and assessing freshness and quality.

4.2.1 MOISTURE CONTENT

Fish almost contain 65% of moisture and it is a vital initial step because fresh fish muscle tightly binds water to its protein structure, making it resistant to expulsion even under high pressure. However, prolonged chilled or frozen storage before moisture analysis weakens this bond, leading to some water loss along with dissolved substances. To remove moisture and low-volatile materials, heating under a partial vacuum at 95-100°C is employed, a method applicable to various fish products including frozen scallops and fish by-products. The moisture content was determined according to the method of Association of Official Analytical Chemistry (AOAC, 1995)

To initiate the moisture determination process, take an empty, sterilized, and dry glass plate which is carefully weighed using a precision weighing machine. Subsequently, a representative portion of each muscle sample *Sphyraena obtusata* and *Psettodes erumei* is placed onto the labeled glass plate, and the combined weight of the plate and the sample is recorded as the wet weight. The oven is then preheated to a temperature of 105°C, and the glass plates with the samples are transferred into the hot air oven. Maintaining a constant temperature, the samples are left overnight until they are thoroughly dried to obtain a consistent dry weight. Once dried, the glass plates with the dried samples are removed from the oven, and the final weight, known as the dry weight, is recorded. This meticulous procedure ensures an accurate determination of moisture content in the fish samples, essential for subsequent analyses and quality assessment (Fig 4.2.1.1 and Fig 4.2.1.2)



(a)

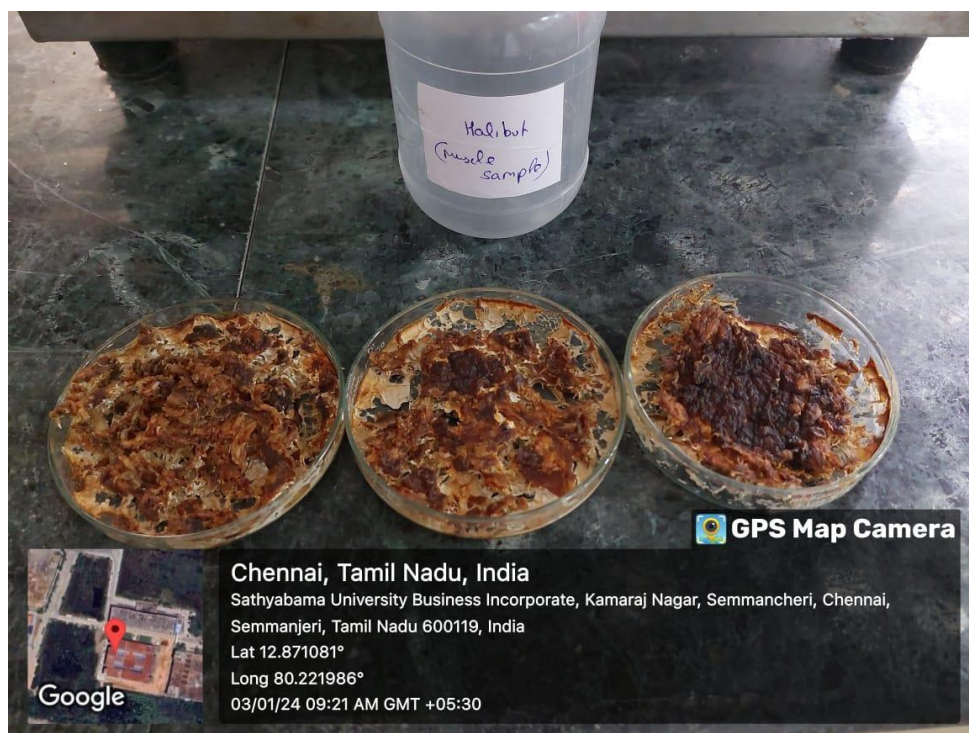


(b)

Fig 4.2.1.1 Wet Sample(a) and Dry Sample(b) of *Sphyraena obtusata*(Barracuda)



(a)



(b)

Fig 4.2.1.2 Wet Sample(a) and Dry Sample(b) of *Psettodes erumei* (Indian halibut)



Fig 4.2.1.3 Dry powdered sample of *Sphyraena obtusata* (Barracuda) and *Psettodes erumei* (Indian halibut)

After completing the calculation use a mixer grinder to homogenize it effectively. Then, securely store the dry powdered sample in a labeled container to maintain its quality for further Proximate analysis.(Fig 4.2.1.3)

4.2.2 DETERMINATION OF PROTEIN

The main principle of Lowry's methodology is that the peptide bond (-CO-NH-) within a polypeptide chain interacts with copper sulfate under alkaline conditions, forming a blue-colored complex. Furthermore, the tyrosine and tryptophan residues present in proteins can lead to the reduction of the phosphomolybdate and phosphotungstate components of the Folin-Ciocalteu reagent, generating bluish products. These reactions collectively contribute to the increased sensitivity of the Folin-Ciocalteu method.

For the preparation, analytical reagent solutions involves the creation of three distinct solutions: Solution A comprises 0.5 g of copper sulfate (CuSO_4) dissolved in 50 mL of distilled water; Solution B consists of 2% sodium potassium tartrate dissolved in 50 mL of distilled water; and Solution C involves dissolving 2% alkaline sodium carbonate in 0.1N sodium hydroxide (NaOH). These solutions are then combined, with 50 mL of Solution A, 0.5 mL of Solution B, and 1 mL of Solution C, to produce the analytical reagent(Fig 4.2.2.1).



Fig 4.2.2.1 Analytical Reagent



Fig 4.2.2.2 Follin Reagent

Additionally, Folin's reagent is prepared by diluting Folin-Ciocalteu reagent with an equal volume of 25ml (Fig 4.2.2.2). For the standard solution, 50 mg of Bovine Serum Albumin (BSA) are dissolved in 50 ml of distilled water in a volumetric flask. From this stock standard, 10 ml are extracted and diluted to 50 ml in another flask to create a working standard solution, where each ml contains 200 mg of protein.

For the sample preparation extract 1 g from both *Sphyraena obtusata* and *Psettodes erumei* and dissolve each in 1 milliliter of distilled water

To initiate the procedure, a series of labeled test tubes are prepared, each containing different volumes (0.2, 0.4, 0.6, 0.8, and 1 ml) of the working standard, designated as S1 to S5 respectively. A test tube with 1 ml of distilled water serves as the blank. Similarly, a triplicate set of test tubes labeled B1,B2, and B3 to H1, H2, and H3 are

filled with 0.5 ml of the fish samples from *Sphyraena obtusata* and *Psettodes erumei* respectively., with the volume adjusted to 1 ml in all tubes

Now ,2.5 ml of the analytical reagent are added to each test tube, including the blank tubes. The contents are mixed thoroughly by vortexing or shaking and left to stand for 10 minutes. Following this, 0.5 ml of Folin's reagent are rapidly added to each tube, with immediate mixing, and the tubes are incubated at room temperature in the dark for 30 minutes.(Fig 4.2.2.3 , Fig 4.2.2.4) .

Finally, the absorbance of the samples is measured at 720 nanometers against the blank. A standard curve is then plotted using the concentration of standard protein along the X-axis and the absorbance at 720 nanometers along the Y-axis.

c



Fig 4.2.2.3 Vortex Fig 4.2.2.4 Sample Incubation Period of *Sphyraena obtusata* (Barracuda) and *Psettodes erumei* (Halibut)

Calculate the Concentration of protein of *Sphyraena obtusata* and *Psettodes erumei* triplicate sample with the help of slope equation.

4.2.3 DETERMINATION OF CARBOHYDRATE

Typically, carbohydrates are often overlooked in analyses due to their relatively low presence, constituting a small percentage of wet mass, usually assumed to be less than 0.5%.

The principle of anthrone methodology is that carbohydrates present in the fish muscle undergo a chemical transformation when treated with concentrated sulfuric acid (H_2SO_4), resulting in the production of furfural. Furfural exhibits a distinctive green color when it reacts with anthrone through a process called condensation.

Anthrone, the reagent in this process, demonstrates reactivity with a wide range of carbohydrates including dextrans, monosaccharides, disaccharides, polysaccharides, starch, gums, and glycosides (Plummer, 2008)

For reagent preparation, anthrone reagent is formulated by dissolving 200 mg of anthrone in 100 ml of concentrated H_2SO_4 . It's essential to prepare this reagent fresh before each use. (Fig 4.2.3.1)



Fig 4.2.3.1 Anthrone Reagent

Standard glucose solution and sample preparation follow a similar protocol as protein estimation

To initiate the procedure, a series of labeled test tubes are prepared, each containing different volumes (0.2, 0.4, 0.6, 0.8, and 1 ml) of the working standard, designated as S1 to S5 respectively. A test tube with 1 ml of distilled water serves as the blank. Similarly, a triplicate set of test tubes labeled B1, B2, and B3 to H1, H2, and H3 are filled with 0.5 ml of the fish samples from *Sphyaena obtusata* and *Psettodes erumei* respectively., with the volume adjusted to 1 ml in all tubes

Then, 4 ml of the anthrone reagent are added to each tube, thoroughly mixed by vortexing. The test tubes are covered with a cotton plug and incubated either at 90 degrees Celsius for 18 minutes or in a boiling water bath for 10 minutes.(Fig 4.2.3.2)



Fig 4.2.3.2 Triplicate sample of *Sphyaena obtusata* and *Psettodes erumei* is kept in Water bath .

After cooling to room temperature, the optical density is measured at 620 nanometers against the blank.

Finally, a standard curve is plotted using the concentration of standard carbohydrate along the X-axis and absorbance at 620 nanometers along the Y-axis. The concentration of carbohydrates in the triplicate samples of *Sphyaena obtusata* and *Psettodes erumei* is calculated using the slope equation derived from the standard curve.

4.2.4 DETERMINATION OF LIPID/FAT CONTENT

The Blight and Dyer method, a straightforward cold lipid extraction technique, was initially devised as a cost-effective approach for extracting lipids from tissues with low lipid content and high water proportion, such as fish muscle. Widely adopted, it bears resemblance to the Folch method, with distinctions primarily lying in solvent ratios. In this method, lipids are extracted from homogenized cell suspensions using a 1:2 (v/v) chloroform/methanol mixture. Subsequently, lipids from the chloroform phase undergo further processing through various procedures. In reagent preparation, 30 ml of chloroform and 30 ml of methanol are prepared. Additionally, a sodium chloride solution is made by adding 0.18 g of NaCl to 20 ml of distilled water. The procedure commences by weighing 0.5 g of fresh minced fish muscle into test tubes labeled as B1 to B3 for *Sphyaena obtusata* and H1 to H3 for *Psettodes erumei*. To each tube, 1 ml of pre-cooled chloroform and 2 ml of methanol are added. Following this, the mouth of each test tube is covered with a cotton plug, and the contents are blended for 2 minutes in a vortex. An additional 1 ml of chloroform is then added to each tube, followed by blending for another 2 minutes. Subsequently, 0.8 ml of freshly prepared sodium chloride solution is added to each test tube. The next step involves moistening Whatman filter paper with water and utilizing it to filter the mixture. Following filtration, two liquid phases are obtained: the upper phase containing methanol and the lower phase containing chloroform. (Fig 4.2.4.1)



Fig 4.2.4.1 Filtration results in two liquid phases of *Sphyaena obtusata* and *Psettodes erumei* .

The upper phase is discarded, and the lower phase is collected using a pipette.(Fig 4.2.4.2)

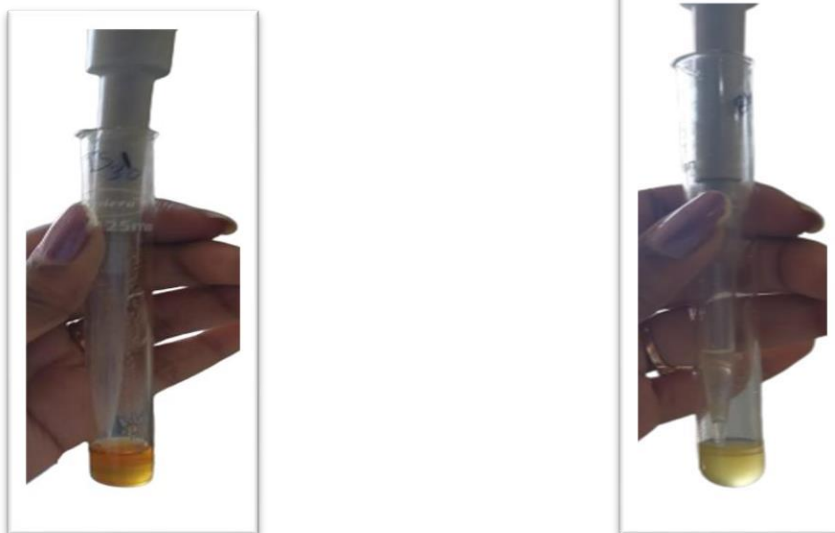


Fig 4.2.4.2 Separation of two liquid phases of *Sphyraena obtusata* and *Psettodes erumei* .

Next, small aluminum dishes are prepared and pre-weighed. The obtained chloroform phase is added to the dishes, and the dishes are re-weighed with the wet sample. They are then placed on a hot metal plate or a low RPM set magnetic stirrer for 10 minutes, followed by 15 minutes in a hot air oven.(Fig 4.2.4.3)



Fig 4.2.4.3 RPM set magnetic stir of *Sphyraena obtusata* and *Psettodes erumei* .

4.3 EXTRACTION OF ACID SOLUBLE COLLAGEN FROM SKIN SAMPLE OF *Sphyraena obtusata* and *Psettodes erumei*

NOTE : To safeguard the integrity of the protein, it's important to maintain a consistently cold temperature of 4°C throughout all stages of the process.

After dissection, the skin samples from *Sphyraena obtusata* and *Psettodes erumei* are manually excised and cut into small pieces which are immersed in cold water at 4°C for 3 days (change water in 2 days). After the desalting process, the skin samples was washed and stored under 80°C until further use.(Fig 4.3.1)



Fig 4.3.1 Desalting

The skin samples from *Sphyraena obtusata* and *Psettodes erumei* were smashed by tissue homogenizer.(Fig 4.3.2)

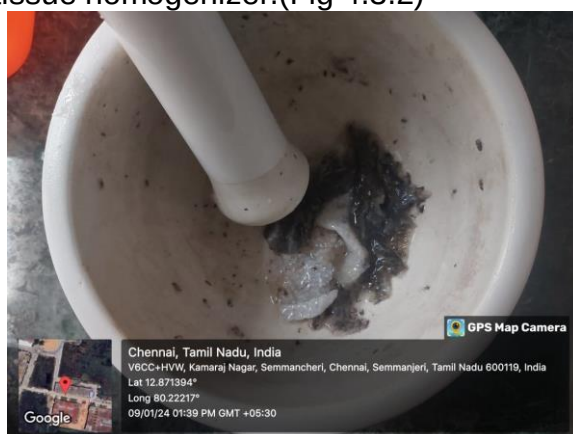


Fig 4.3.2 Tissue Homogenizer and Acetic acid

With the smashed skin sample 0.6M of acetic acid solution was added and kept under stirring at 4°C for 72 hours(Fig 4.3.3)



Fig 4.3.3 Magnetic stir of *Sphyaena obtusata* and *Psettodes erumei* in acetic acid

After 72 hours of gentle stirring, each sample is filtered using cheesecloth and left without any disruptions. Following filtration, a freshly prepared 0.9M sodium chloride solution is gently introduced to the filtered sample, which is then left undisturbed until precipitation occurs(Fig 4.3.4)

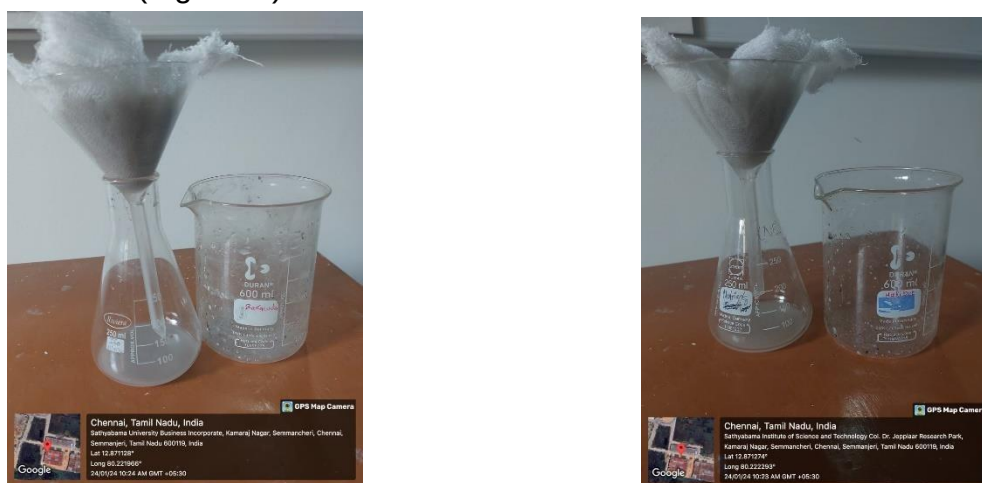


Fig 4.3.4 Filtration of *Sphyaena obtusata* and *Psettodes erumei* .

After filtration, the sample undergoes centrifugation at 4,000 rpm for 15 minutes. The resulting pellet is transferred to a petri dish and stored in a deep freezer at -80°C before being processed in a lyophilizer(Fig 4.3.5)



Fig 4.3.5 Centrifugation of Acid soluble Collagen *Sphyraena obtusata* and *Psettodes erumei* .

4.2.1 LYOPHYLISOR

To transform the semi-liquid acid-soluble collagen into a powdered form and ensure its stability , the process of lyophilization, also known as freeze-drying, was employed. This technique operates on the principle of removing water from a substance through

sublimation, wherein water transitions directly from a solid to a gas phase without becoming a liquid.

The lyophilization process for the collagen took approximately 20 hours to achieve the desired powdered form. During this duration, the ASC of was subjected to freezing and subsequent vacuum drying, allowing the water content to sublime directly, leaving behind a dry, powdered product. This ensures the stability and longevity of the collagen, making it suitable for storage and use under cold conditions.(Fig 4.3.1.1)

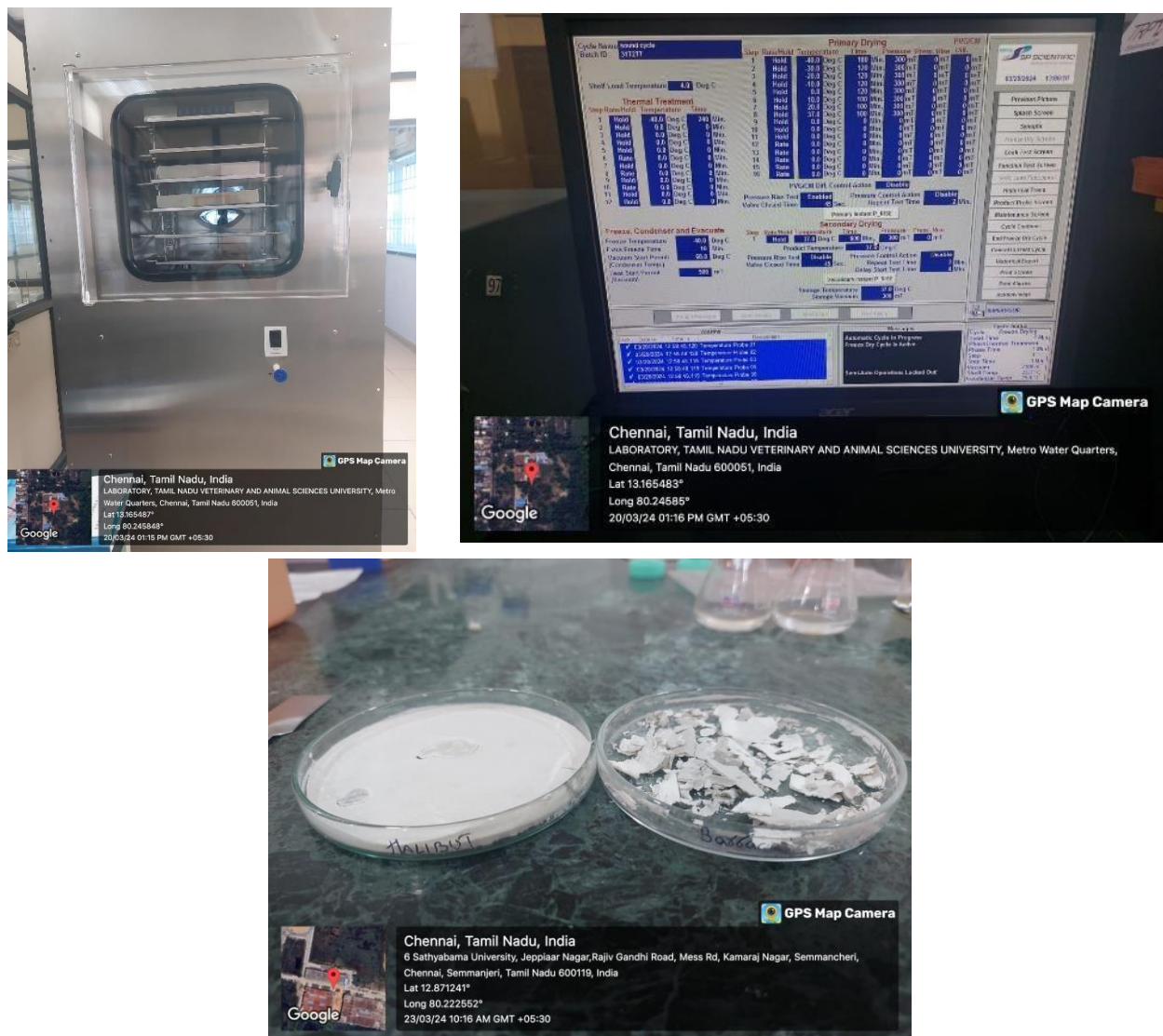


Fig 4.3.1.1 Lyophilised sample of *Sphyraena obtusata* and *Psettodes erumei* .

Following lyophilization, we obtain the powdered form of Acid Soluble Collagen (ASC). It's imperative to ascertain the protein concentration in the obtained ASC. To achieve this, we utilize the Bradford assay, which provides a reliable measure of protein concentration. Additionally, we employ SDS-PAGE to determine the size of the proteins present within the ASC sample.

4.3.2 BRADFORD ASSAY

For proteins, PBS (phosphate-buffered saline) serves as an effective buffer solution. To prepare PBS, combine 0.72g of sodium phosphate dibasic, 0.12g of potassium dihydrogen phosphate, 4g of sodium chloride, and 0.1g of potassium chloride in 250 ml of water. Stir the mixture using a magnetic stirrer for 5 minutes and adjust the pH to 7.4. Use a standard 500ml conical flask to make up the PBS.(Fig 4.3.2.1)

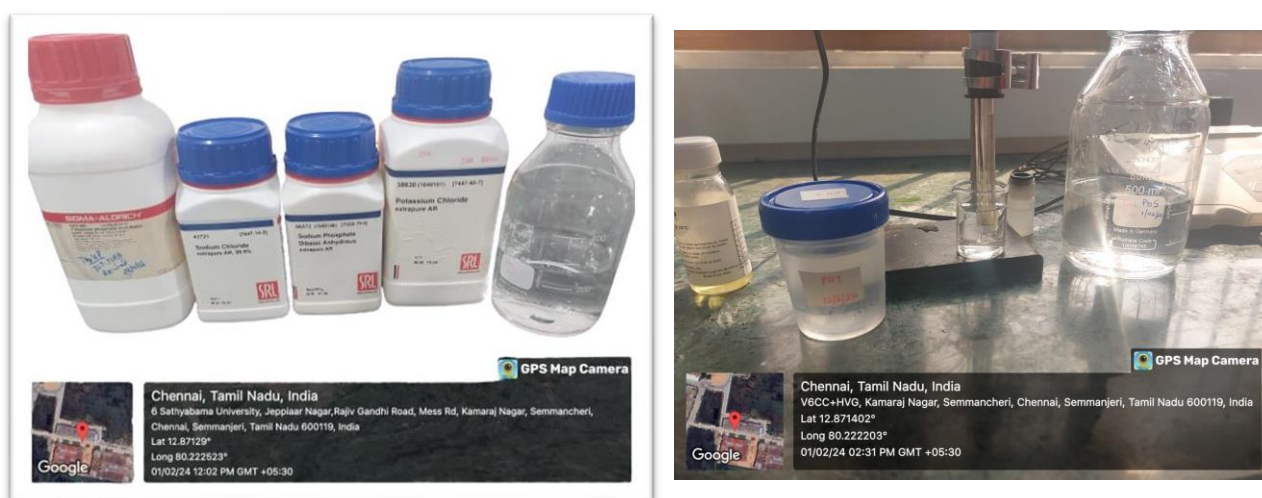


Fig 4.3.2.1 PBS Reagent and Ph meter

To prepare the Bradford reagent, combine 50ml of orthophosphoric acid, 25ml of 95% ethanol, and 0.05g of Coomassie Brilliant Blue in a dark reagent beaker. Add 100ml of water to the mixture. Stir the solution overnight. Afterward, make up the mixture to 500ml, filter the Bradford reagent in another dark flask container using pre-weighed Whatman paper filters by maintaining it in a dark room. Store the reagent at 4°C for future use.(Fig 4.3.2.2 and Fig 4.3.2.3)

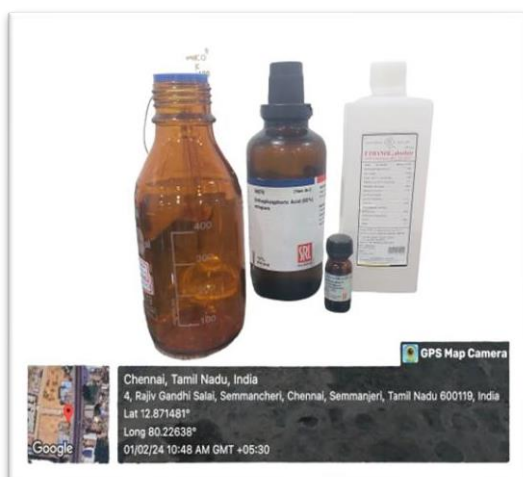


Fig 4.3.2.2 Bradford Reagents



Fig 4.3.2.3 Magnetic stir



Fig 4.3.2.3 Filtration

To prepare BSA for standardization, 2mg of BSA is dissolved in 2 mL of distilled water in an Eppendorf tube. For sample preparation, 0.001g of acid-soluble collagen extracted from *Sphyaena obtusata* (Barracuda) and *Psettodes erumei* (Indian halibut) is individually mixed with 1ml of distilled water. The mixture undergoes gentle

pulverization under cold conditions using a mortar and pestle, and the resulting solution is collected in Eppendorf tubes. The procedure involves pipetting out volumes of 10 μ L, 20 μ L, 40 μ L, 80 μ L, 160 μ L, and 320 μ L of the working standard labeled as S1, S2, S3, S4, S5, and S6 respectively into labeled test tubes. A tube containing 1 mL of distilled water serves as the blank. Then, 50 μ L of the extracted acid-soluble collagen from *Sphyaena obtusata* and *Psettodes erumei* is added to the test tubes labeled B and H respectively. Finally, PBS is added to each test tube to make up the solution to 1ml. Now add 5 ml of Bradford reagent and maintain this process in a dark room and incubate for 10mins Draw a standard curve with absorbance at 595 nm in Y-axis against protein concentration at X-axis. Determine the ASC concentration from the standard curve.

4.3.3 SDS PAGE

SDS-PAGE, or Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis, is a widely used technique in biochemistry and molecular biology for separating proteins based on their molecular weight. To prepare Arcrylamide 30%, you'll need to mix 30 g of Arcrylamide and 0.8 g of Bis- Arcrylamide in 30 ml of distilled water. For the 1.5 molar Tris buffer, dissolve 18.17 g of Tris buffer in 50 ml of water . Additionally, prepare a 0.5 molar Tris buffer by dissolving 6.05 g of Tris buffer in 50 ml of water. Keep them in a steric environment. Once the homogeneous mixture is achieved, use a pH meter to measure the pH values. Adjust the pH to 8.8 for the 1.5 M Tris buffer solution and 6.8 for the 0.5 M Tris buffer solution. Lastly, for the SDS 10%, mix 2.5 g of SDS with 25 ml of water. Tank buffer is preaperded with the help of sds, tris hcl and glycine. Prepare Ammonium Perusulfate freshly of 0.1g in 1ml water. (Fig 4.3.3.1)



Fig 4.3.3.1 SDS reagent

Prepare the stacking and resolving gel for SDS-PAGE in 50ml of eppendorf using the provided table as a reference.

Clean the glass plates and spacers of the gel casting unit with deionized water and ethanol. Assemble the plates with the spacers on a stable, even surface.

Table 4.3.3.1 Restocking and Stocking GEL

RESTICKING GEL		STOCKING GEL	
acrylamide %	10%	Water	2.93ml
Water	3.8ml	acrylamide/Bis (30/0.8/w/v)	0.69ml
acrylamide/Bis (30/0.8/w/v)	3.4ml	1.5M tris buffer (ph 8.8)	1.3ml
1.5M tris buffer (ph 8.8)	2.6ML	10% (w/v) SDS	50μL
10% (w/v) SDS	0.1ML	10% (w/v) APS	50μL
10% (w/v) APS	100μL	TEMED	10μL/5μL
TEMED	10μL		

Pour the freshly prepared Restocking solution about 4ml in the plates assembled with spacers. To maintain an even and horizontal restocking gel surface, overlay the surface with water or isopropanol. Allow the gel to set for about at room temperature. Now add freshly prepared stocking gel solution about 4 ml or until it over fills in the plate. Discard the overlayed water or isopropanol on the resolving gel. Insert the comb immediately ensuring no air bubbles are trapped in the gel or near the wells. Allow the gel to set at room temperature. Prepare marker. For sample preparation acid-soluble collagen extracted from *Sphyaena obtusata* (Barracuda) and *Psettodes erumei* (Indian halibut) follow the same prepartion as bradford assay. Now place the centrifuge tube in sonicator for 5 mins. (Fig 4.3.3.2)



Fig 4.3.3.2 Sonication of Acid Soluble Collagen of *Sphyraena obtusata* and *Psettodes erumei*

After sonication the sample is let to cool for 2 mins then , in the first set, labeled B1 and H1, 10 μ L of gel loading solution are added along with 50 μ L of the sample solution. In the second set, labeled B2 and H2, 25 μ L of gel loading solution and 25 μ L of each respective sample are added. Subsequently, all tubes are submerged in a water bath for a 5-minute incubation period. Following incubation, the contents of each tube are carefully poured into their designated wells on the gel. The SDS station is then filled with tank buffer, and the gel is allowed to run for a duration of 3 hours.(Fig 4.3.3.3)



Fig 4.3.3.3 Gel Electrophoresis of Acid Soluble Collagen of *Sphyraena obtusata* and *Psettodes erumei*

Once the electrophoresis process is complete, the gel is gently removed and placed in a rocking chair with Coomassie stain dye for overnight incubation, facilitating visualization of the separated protein bands. Staining (Fig 4.3.3.4)



Fig 4.3.3.4 Staining of *Acid Soluble Collagen* of *Sphyraena obtusata* and *Psettodes erumei* In gel rocker

CHAPTER 5

RESULT AND DISCUSSION

5.1 MOISTURE CONTENT

Table 5.1.1 WET AND DRY WEIGHT

	WET WEIGHT	DRY WEIGHT
Barracuda sample 1 (B1)	79g	32g
Barracuda sample 2 (B1)	103g	34g
Barracuda sample 3 (B1)	106g	22g
Barracuda total (B)	288g	88g
Halibut sample 1 (H1)	95g	22g
Halibut sample 2 (H1)	78g	31g
Halibut sample 3 (H1)	105g	27g
Halibut Total (H)	278g	80g

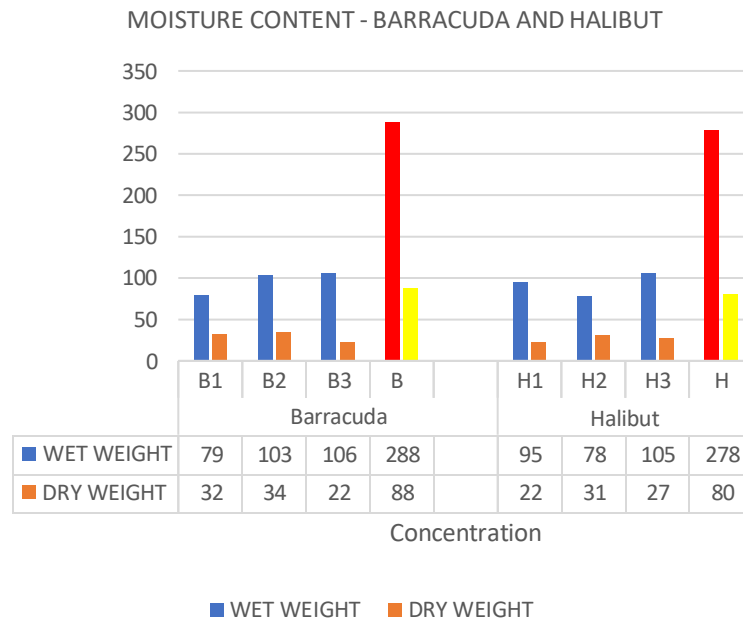


Fig 5.1.1 MOISTURE CONTENT of *Sphyraena obtusata* AND *Psettodes erumei*

With the help of the table (**Table 5.1.1**) the graph is plotted which reveals the wet and dry weights of *Sphyraena obtusata* (Barracuda) and *Psettodes erumei* (Indian halibut). Utilizing these measurements, we determined the moisture content employing the formula (**Fig 5.1.1**)

$$\% \text{ moisture} = 100 \times (\text{wet weight} - \text{dry weight}) / (\text{wet weight} - \text{initial weight})$$

After applying the respective values from the table, we calculated the moisture percentage to be 66.51% for *Sphyraena obtusata* (Barracuda) and 52.50% for *Psettodes erumei* (Indian halibut). The *Sphyraena obtusata* (Barracuda) exhibited 66.51% moisture content, which deviates from the previously reported 73.79% by etPalani,2014 This variance can likely be attributed to factors such as fish length and environmental conditions. Similarly, the moisture value for *Psettodes erumei* closely resembled that reported by Mannan *et al.*, 2023 suggesting a consistency in the observed moisture content

5.2 LOWRYS METHEDOLLOGY.

Table 5.2.1 OD VALUE AT 720nm

	Absorption
Barracuda sample 1 (B1)	0.156nm
Barracuda sample 2 (B2)	0.153nm
Barracuda sample 3 (B3)	0.149nm
Barracuda Avg (B)	0.153nm
Halibut sample 1 (H1)	0.329nm
Halibut sample 2 (H2)	0.335nm
Halibut sample 3 (H3)	0.334nm
HalibutAvg (H)	0.334nm

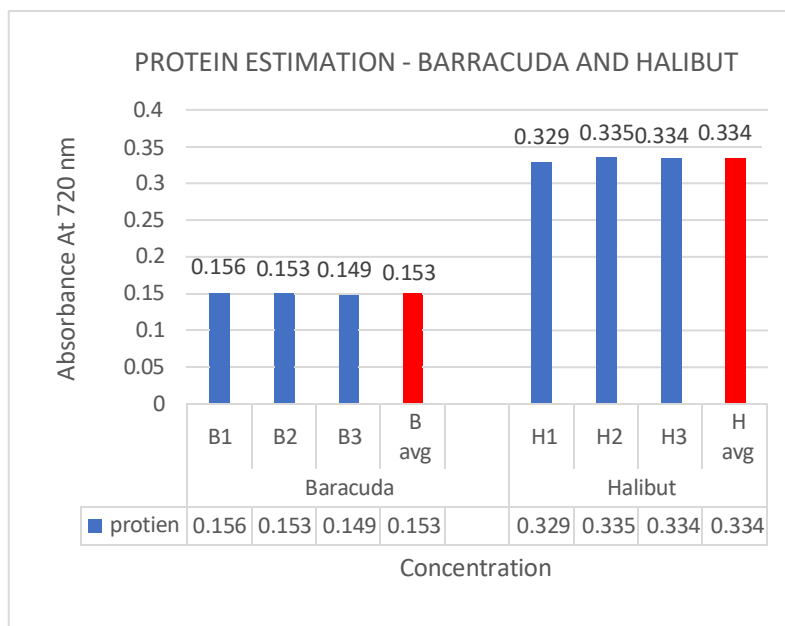


Fig 5.2.3 Lowrys – *Sphyraena obtusata* and *Psettodes erumei*

The results of the proximate analysis reveal notable differences in the protein concentration between exhibited a protein concentration of 0.964 mg/ml, while *Psettodes erumei* displayed a substantially higher protein concentration of 3.111 mg/ml With the help of UV-SPECTROMETER .

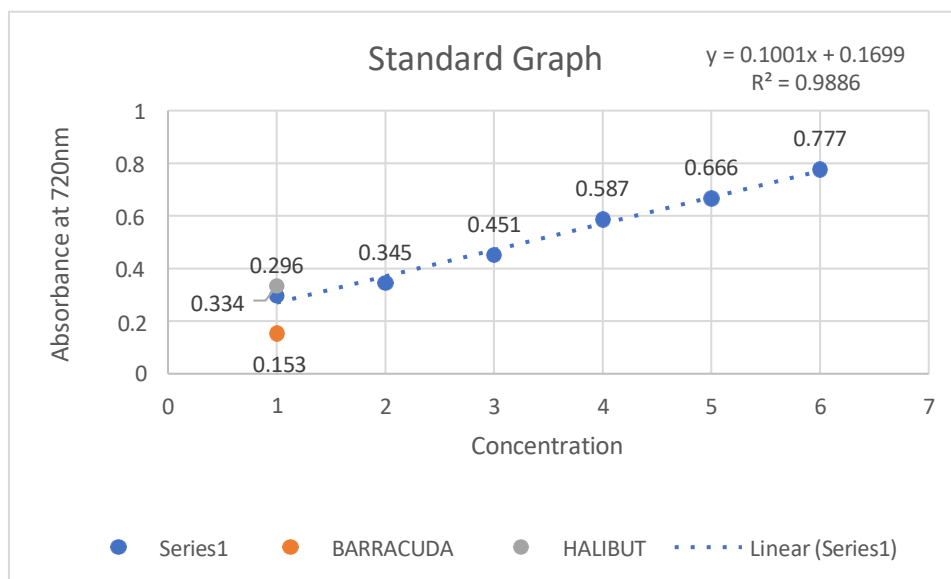


Fig 5.2.3 Graphical Representation of *Sphyraena obtusata* and *Psettodes erumei*

These findings are consistent with previous research conducted by K. Sujatha *et al.* (2013), who also reported a similar protein concentration in *Sphyaena obtusata*. Additionally, the high protein concentration observed in *Psettodes erumei* aligns with findings from Jeong Dae Kim and Santosh Lall ,(2000), who concluded that *Psettodes erumei* is one of the great producers of protein among flat fishes

5.3 ANTHRON METHEDOLOGY

Table 5.3.1 OD VALUE AT 630nm

Barracuda sample 1 (B1)	0.039
Barracuda sample 2 (B1)	0.037
Barracuda sample 3 (B1)	0.041
Barracuda total (B)	0.039
Halibut sample 1 (H1)	0.038
Halibut sample 2 (H1)	0.044
Halibut sample 3 (H1)	0.049
HalibutAvg (H)	0.043

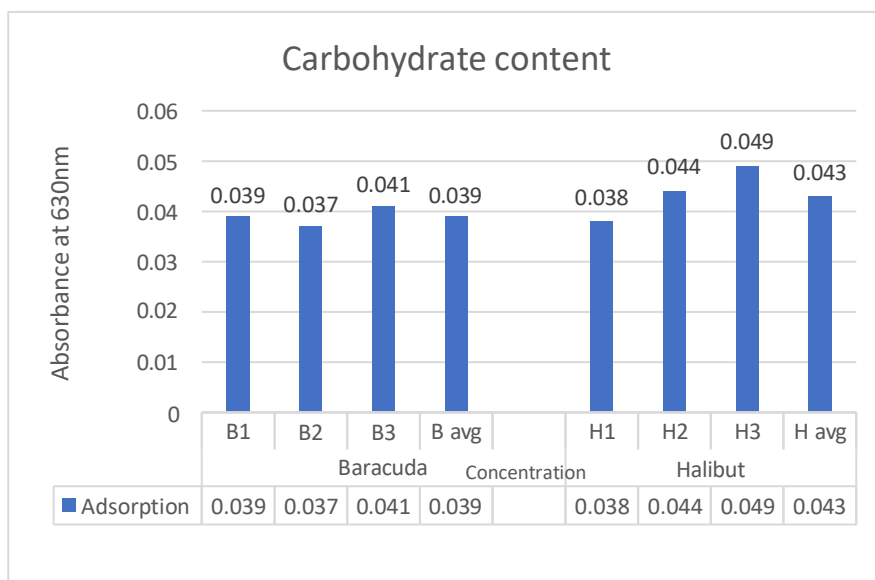


Fig 5.3.2ANTHRON – *Sphyaena obtusata* and *Psettodes erumei*

Carbohydrate concentration was calculated to be 0.003 mg/ml for *Sphyaena obtusata* while it measured 0.001 mg/ml for *Psettodes erumei*

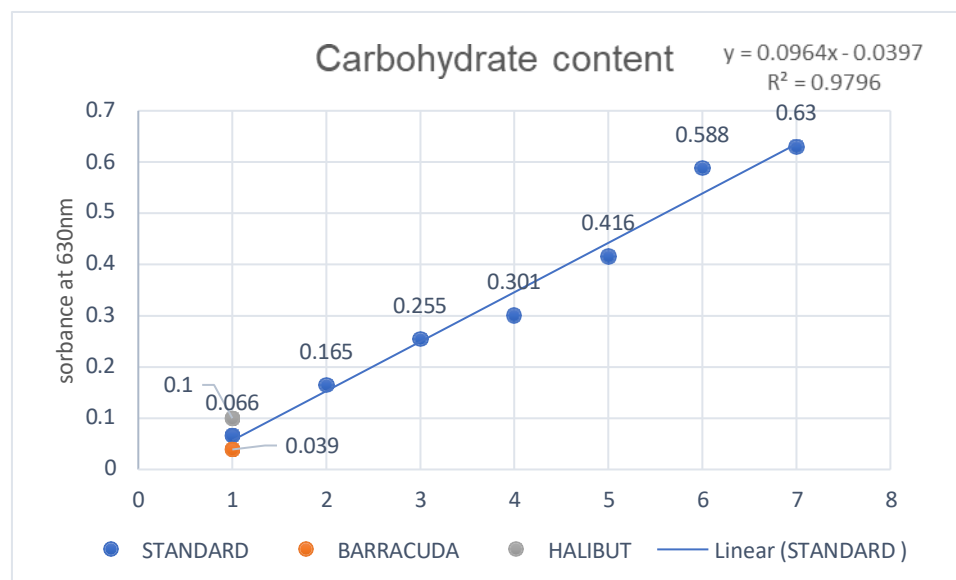


Fig 5.3.3 Graphical Representation of *Sphyraena obtusata* and *Psettodes erumei*

These findings align with the ecological characteristics of the respective species. *Sphyraena obtusata*, being a pelagic fish, likely exhibits a higher carbohydrate concentration due to its active swimming behavior and potentially varied diet, which may include plankton and other carbohydrate-rich organisms found in open water habitats. On the other hand, *Psettodes erumei*, a demersal fish, inhabits beneath environments where carbohydrate-rich food sources might be comparatively scarce. Therefore, its lower carbohydrate concentration is expected, reflecting adaptations to its ecological niche.

5.4 LIPID ESTIMATION

Table 5.4.1 WET ,DRY WEIGHT AND LIPID CONTENT OF *Sphyraena obtusata* AND *Psettodes erumei*

	WET WEIGHT	DRY WEIGHT	LIPID CONTENT
Barracuda sample 1 (B1)	0.2g	0.21g	0.01g
Barracuda sample 2 (B2)	0.22g	0.2g	0.02g
Barracuda sample 3 (B3)	0.19g	0.17g	0.02g

Barracuda (B)	0.2g	0.19g	0.02g
Halibut sample 1 (H1)	0.16g	0.14g	0.02g
Halibut sample 2 (H2)	0.18g	0.17g	0.01g
Halibut sample 3 (H3)	0.14g	0.13g	0.01g
HalibutAvg (H)	0.16g	0.14g	0.01g

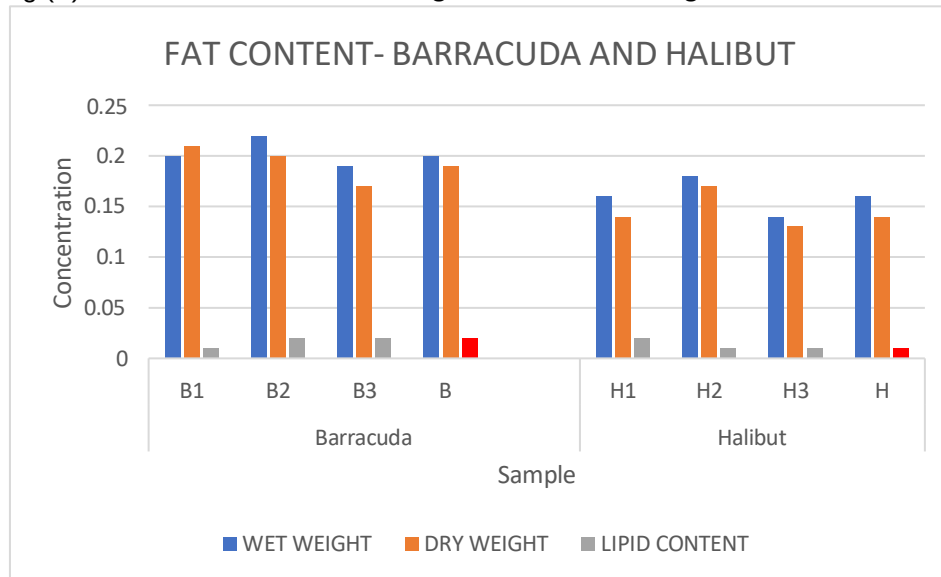


Fig 5.4.2 BLIGH AND DYER – *Sphyraena obtusata* and *Psettodes erumei*

The results of the proximate analysis reveal differences in lipid concentrations between *Sphyraena obtusata* (Barracuda) and *Psettodes erumei* (Indian halibut) muscle samples. *Sphyraena obtusata* exhibited a lipid concentration of 0.02 mg/ml, while *Psettodes erumei* displayed a slightly lower concentration of 0.01 mg/ml.

Comparative studies with other fish species support these observations. For example, research by Pretty, J *et al.*, 2018 found that lipid concentrations in pelagic fish species were significantly higher compared to demersal species. This trend may be attributed to differences in feeding habits, metabolic rates, and environmental factors influencing lipid accumulation in fish muscle.

5.5 BRADFORD ASSAY

The Bradford assay was employed to quantify the acid-soluble collagen content in the samples under investigation. Absorbance readings were recorded at the appropriate wavelength for each sample, and collagen concentrations were determined using a standard curve derived from known collagen concentrations.

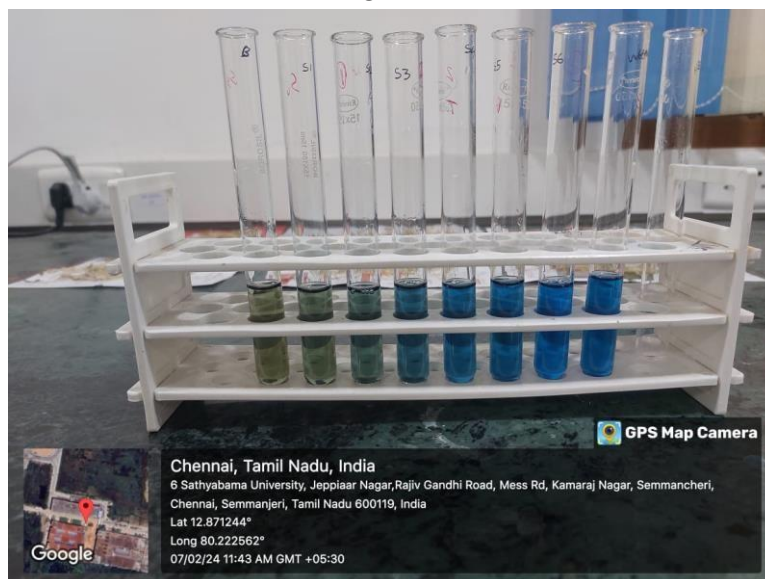


Fig 5.5.1 Bradford Assay of standard vs asc of *Sphyraena obtusata* and *Psettodes erumei*

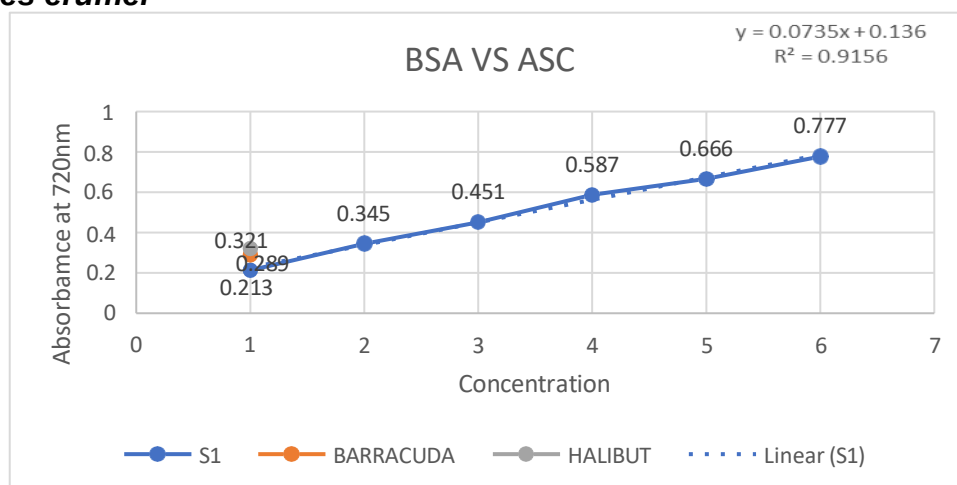


Fig 5.5.2 presents the graphical collagen concentrations for the acid-soluble collagen in *Sphyraena obtusata* and *Psettodes erumei*

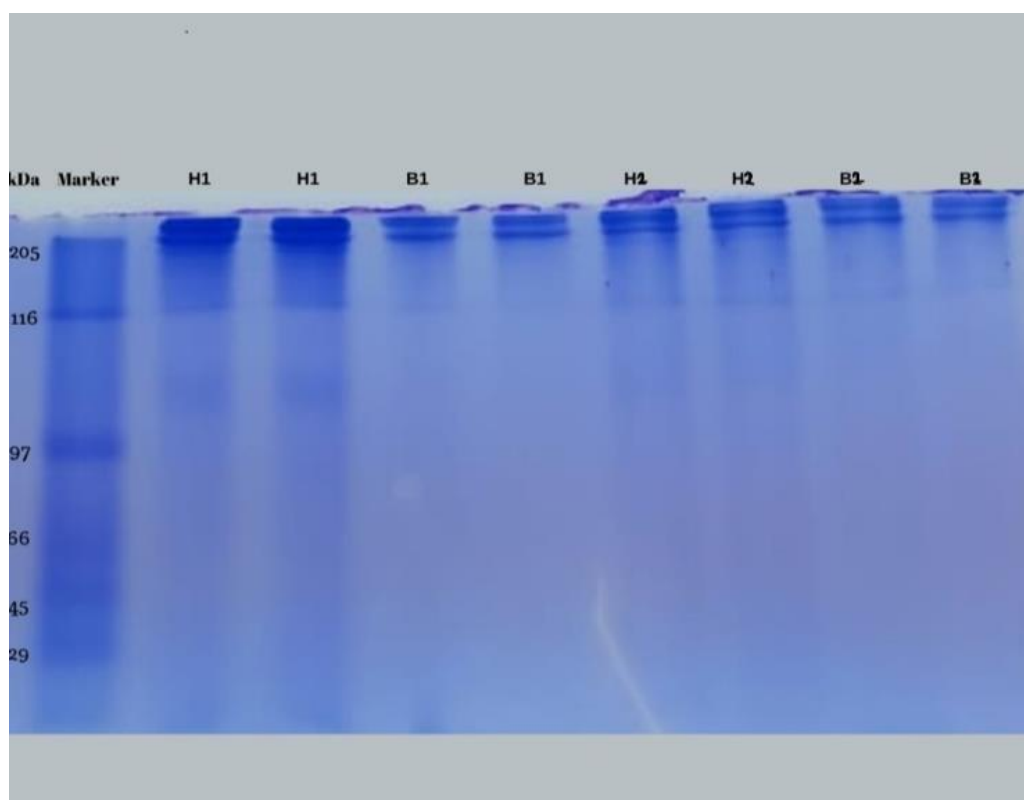
Sphyraena obtusata exhibited the collagen concentration at 2.081 mg/ml while *Psettodes erumei* had a highest collagen concentration of 2.517mg/ml. These findings suggest that there may be small differences in the collagen content of the samples, potentially attributable to factors such as species variation or sample processing methods.

Despite these limitations, the results of the Bradford assay provide valuable insights into the acid-soluble collagen content of *Sphyraena obtusata* and *Psettodes erumei*. The observed differences in collagen concentration highlight the importance of further investigation into the factors influencing collagen content in these samples, as well as their implications for applications in various fields such as biomedicine and tissue engineering.

Moving forward, future studies could explore additional assays or techniques to validate the findings of this assay and provide a more comprehensive understanding of the collagen composition in the samples. Overall, the results of the Bradford assay contribute to our understanding of acid-soluble collagen content in *Sphyraena obtusata* and *Psettodes erumei* under investigation and pave the way for further research in this area.

5.6 SDS-PAGE

SDS-PAGE analysis was conducted to characterize the protein composition of *Sphyraena obtusata* and *Psettodes erumei* and assess the presence of collagen. Electrophoretic separation of proteins was achieved using polyacrylamide gel, with subsequent Coomassie staining for visualization. Fig5.6.1 illustrates the protein bands obtained from the SDS-PAGE analysis of the samples.



Upon examination of the gel, distinct protein bands were observed, indicating the presence of various proteins in *Sphyraena obtusata* and *Psettodes erumei*. A prominent band corresponding to the molecular weight of collagen was identified, confirming the presence of collagen in the samples with higher molecule. The concentrations H1 and H2 in *Psettodes erumei* exhibit a notably darker dye appearance compared to B1 and B2 of *Sphyraena* species. Our analysis reveals a predominant molecular weight of 205 kDa in collagen, consistent with the findings reported by Manal Shalaby et al. (2019) regarding *Mugil cephalus* fish and Liu, Dasong, et al. (2012). This convergence of results underscores the robustness of the observed 205 kDa molecular weight in collagen across different fish species and studies.

Moving forward, future studies could explore additional techniques, such as Western blotting or mass spectrometry, to further characterize the identified protein bands and elucidate their specific identities. Overall, the results of the SDS-PAGE analysis contribute to our understanding of the protein composition of *Sphyraena obtusata* and *Psettodes erumei*.pave the way for future research in this area.

CHAPTER 6

SUMMARY AND CONCLUSION

In summary, our study focused on the proximate composition analysis and collagen isolation from pelagic (*Sphyraena obtusata*) and demersal (*Psettodes erumei*) fish species found in the coastal region of Chennai. Through proximate analysis, we determined the moisture, protein, lipid, and carbohydrate contents of these fish species, providing essential nutritional information. Additionally, collagen was successfully isolated from fish skin, highlighting the potential utilization of fish by-products in various industrial applications. This research contributes to our understanding of the nutritional value of these fish species and underscores the importance of utilizing fish resources efficiently in coastal regions like Chennai.

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