LEVEL OF HYDROXYL RADICAL SCAVENGING ACTIVITY OF CURED AND UNCURED BOILED SCOMBER SCROMBUS (SCUBIA) OIL

BY

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CERTIFICATION

We the undersigned hereby certify that Mr. Eyayomor Miracle Ejaife carried out this work, in the Department of Medical Biochemistry, University of Benin, Benin City and we approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc.) in Medical Biochemistry.

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DEDICATION

This work is dedicated to God Almighty for His goodness and mercy upon my life, for his guidance, wisdom and direction during the course of this work. I dedicate this research work to my parents, for their unending support so far which led to the success of this research.

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ABSTRACT

Lipids, including fats, sterols, and fat-soluble vitamins, play essential roles in energy storage, cell signaling, and cellular structure, making them vital to biological systems and industries such as food, cosmetics, and nanotechnology. Seafood, particularly fish like *Scomber scombrus* (Atlantic mackerel), is rich in omega-3 fatty acids, which provide numerous health benefits, including protection against heart diseases and oxidative stress. This study investigates the hydroxyl radical scavenging activity of fish oil extracted from *Scomber scombrus* using various preparation methods (raw, marinated, boiled, and fried). The antioxidant potential of the oil, assessed through its ability to inhibit malonaldehyde (MDA) formation via the Fenton reaction, revealed that raw cured samples exhibited the highest scavenging activity, while boiled samples showed the lowest. These findings highlight the benefits of curing to preserve antioxidant properties in fish oil and suggest its potential application in mitigating oxidative stress-related diseases. Additionally, the research underscores the importance of lipids and omega-3s in seafood quality, preservation, and overall human health. Further exploration of cooking methods and antioxidant stability is recommended to enhance the nutritional value of seafood.

CHAPTER ONE

1.0 INTRODUCTION

Lipids constitute a diverse group of organic compounds, encompassing fats, waxes, sterols, fat-soluble vitamins (such as A, D, E, and K), monoglycerides, diglycerides, phospholipids, and others. Their roles include energy storage, signaling, and serving as structural components of cell membranes (Subramaniam *et al.,* 2011). Lipids find applications in industries such as cosmetics, food, and nanotechnology (Mashaghi *et al.,* 2013).

Defined broadly, lipids are hydrophobic or amphiphilic molecules, allowing them to form structures like vesicles, liposomes, or membranes in aqueous environments. They originate from two biochemical subunits: ketoacyl and isoprene groups. This categorization yields eight main lipid types: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, polyketides, sterol lipids, and prenol lipids(LIPID MAPS, 2023).

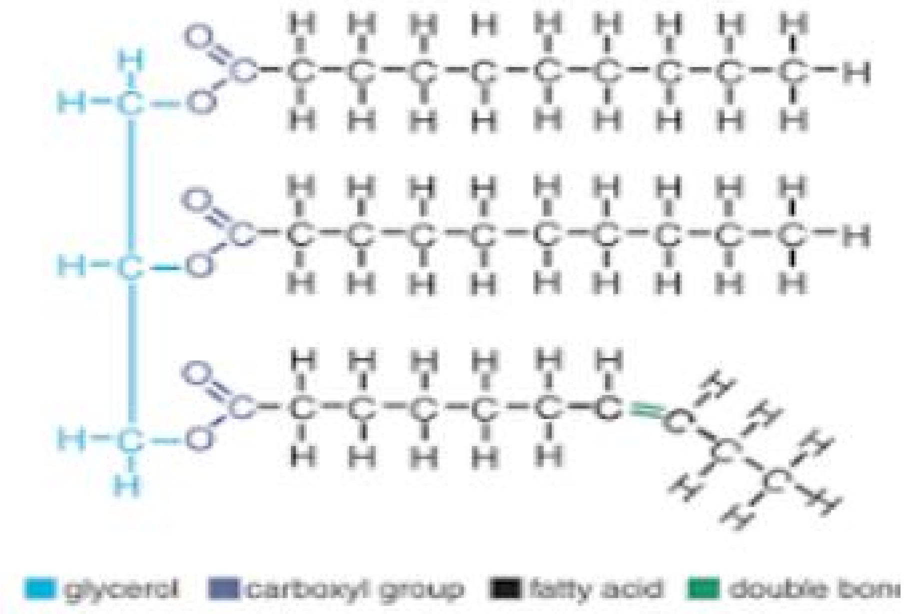

Fig 1.1 Structure of Lipid

While the term "lipid" is often used interchangeably with "fats," fats specifically refer to triglycerides, a subgroup of lipids. Lipids also include molecules like fatty acids and their derivatives (such as tri-, di-, monoglycerides, and phospholipids), as well as sterol-containing metabolites like cholesterol. Despite mammals, including humans, utilizing various biosynthetic pathways for lipid breakdown and synthesis, some essential lipids cannot be produced internally and must be acquired through diet. Lipids are essential components in biological systems, serving vital functions such as storing energy, constructing cell membranes, acting as signaling molecules, and providing insulation and protection for organs(Britannica, 2024).

Seafood encompasses any form of sea life regarded as food by humans, prominently including fish and shellfish. Fish are aquatic vertebrates that lack limbs with digits, use gills to breathe, and have heads protected by hard bone or cartilage skulls. Shellfish, which include various species of mollusks (such as clams, oysters, and mussels) and crustaceans (like shrimp, crabs, and lobsters), are also a major category of seafood.

Seafood can be broadly categorized into several types based on habitat and species. Fish are divided into pelagic fish, which live and feed near the sea's surface (e.g., tuna, mackerel), demersal fish that inhabit the sea floor (e.g., cod, flatfish), diadromous fish that migrate between sea and freshwater (e.g., salmon, eels), and freshwater fish that live in rivers and lakes (e.g., tilapia, catfish). Shellfish include mollusks such as bivalves (e.g., oysters, mussels), gastropods (e.g., snails, whelks), and cephalopods (e.g., squid, octopus) (Merriam-Webster's, 2023). Crustaceans, another significant group, consist of shrimp, crabs, lobsters, and krill (Hoa and Sorgeloos, 2015).

Seafood is sourced from both wild-caught and aquaculture methods. Wild-caught seafood is harvested directly from oceans, rivers, and lakes, while aquaculture involves farming species under controlled conditions, such as marine aquaculture for salmon and oysters, and freshwater aquaculture for species like tilapia and carp. Sustainable practices in both methods are crucial to ensuring the long-term viability of seafood resources.

Hydroxyl radicals, often called the "detergent of the cell," play a crucial role in biological processes but also pose a significant health risk. Originating from metabolic activities such as cellular respiration, these highly reactive molecules readily interact with cellular components, leading to oxidative stress. This stress disrupts cellular functions and contributes to various diseases like cancer, cardiovascular issues, neurodegenerative disorders, and accelerates aging.

Their impact is far-reaching. Hydroxyl radicals cause DNA damage, potentially fueling cancer by inducing mutations. They also initiate lipid peroxidation, compromising cell membrane integrity, particularly detrimental in lipid-rich tissues like the brain. Additionally, hydroxyl radicals trigger inflammatory responses, leading to chronic inflammation, a precursor to autoimmune diseases, cardiovascular problems, and neurodegenerative conditions (Chatterjee, 2016).

Despite their detrimental effects, the body has antioxidant defenses to neutralize hydroxyl radicals. These defenses, sourced internally and externally, mitigate oxidative damage and protect cells. However, an imbalance or excessive production of hydroxyl radicals can overwhelm these defenses, leading to oxidative stress and associated health issues. Maintaining a delicate balance between hydroxyl radical production and antioxidant defenses is essential for overall health, as their dysregulation can wreak havoc on cellular structures, DNA, and lipids, and provoke inflammation (Lipinski, 2011).

Fish, renowned for its nutritional richness, provides high-quality protein, essential minerals, and vitamins E and A. Its omega-3 polyunsaturated fatty acids (PUFA), particularly EPA and DHA, offer protection against ailments like colon cancer, heart diseases, and immune disorders. Experts recommend including fish in the diet twice a week for these benefits (Arab-Tehrany *et al.,* 2012).

Rich in EPA and DHA, fish oil acts as a potent antioxidant. Despite their susceptibility to oxidation, these fatty acids possess antioxidant properties that safeguard cells from oxidative damage and maintain cell membrane integrity. Additionally, they exhibit anti-inflammatory traits, crucial for combating oxidative stress-induced tissue damage. Paired with antioxidants like vitamin E and astaxanthin, fish oil enhances its protective benefits

These antioxidative properties contribute to various health benefits, supporting cardiovascular, cognitive, and joint health, and reducing the risk of conditions like heart diseases and cognitive decline. Fish, a natural source of antioxidants like vitamin E and peptides, not only inhibits lipid oxidation but also enhances consumer health. Although limited, exploring seasonal effects on fish fillet extracts' antioxidant potential remains an unexplored area (Zzaman *et al.,* 2014).

1.2 Aim of Study

The objective of this research is to assess the antioxidant capacity of oil extracted from cured and uncured boiled fish (Scubia) by examining its hydroxyl radical scavenging activity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Titus (*Scomber scombrus*)

The Atlantic mackerel, known by various names such as Boston, Norwegian, or Scottish mackerel, is a widely distributed species inhabiting temperate waters across the Mediterranean Sea, Black Sea, and northern Atlantic Ocean. These fish typically form large shoals in the epipelagic zone, residing at depths of up to 200 meters, and migrate closer to shore during warmer months, retreating to deeper waters as fall and winter approach (Froese et al., 2017).

Fig 2.1 Titus (*Scomber scombrus*)

Physical Description

Atlantic mackerel are characterized by their elongated bodies, which are steel-blue on top with wavy black stripes, and a silvery-white underside. They possess two widely spaced spiny dorsal fins, two pectoral fins, and several small finlets. Mature individuals generally reach about 30 cm, although some can grow up to 60 cm and weigh as much as 3.4 kg (Froese et al., 2017).

Reproduction

Reproductive activity in Atlantic mackerel is oviparous, occurring near coastal areas during spring and summer. Females can produce up to 450,000 eggs, and juveniles typically reach sexual maturity around two years of age, with a lifespan of up to 17 years. This species is commercially significant for its flavorful and oil-rich meat, which is abundant in omega-3 fatty acids and other nutrients. Globally, nearly one million tonnes of Atlantic mackerel are harvested annually, primarily sold fresh, frozen, smoked, or canned. Despite significant commercial exploitation, the species is classified as Least Concern by the IUCN, reflecting a stable population (Da Ros et al., 2023).

Taxonomy and Distribution

The species was first described by Carl Linnaeus in 1758, receiving the scientific name *Scomber scombrus*, derived from the Greek term for mackerel. Despite its wide distribution, genetic studies reveal no significant differences among eastern Atlantic populations, although some divergence exists on a transatlantic scale. The Atlantic mackerel inhabits waters from Labrador, Canada, to North Carolina in the western Atlantic, and from Iceland and Norway to Mauritania in the eastern Atlantic, including the Mediterranean, Black, and Baltic Seas (Abdussamad et al., 2016).

Behavior and Feeding

These fast-moving fish are constantly swimming to ensure adequate oxygenation and form large schools near the surface, particularly during feeding times. Their diet primarily consists of copepods, with *Calanus finmarchicus* being a key component. Spawning occurs in the spring and summer, with eggs maturing in batches over several days. Larvae develop through three stages before transitioning into juvenile forms, beginning schooling behavior around 50 mm in length.

Commercial Importance and Conservation

The Atlantic mackerel is crucial to various Atlantic fisheries, with the UK and Norway being major contributors to global catches. The fish's meat is known for its distinct flavor, being red on the outside and white on the inside, and it is high in oils, vitamins, and omega-3 fatty acids while being relatively low in mercury compared to other mackerels (FishChoice Inc., 2013).

Despite its robust population status, the IUCN advises continued monitoring due to potential impacts from climate change. To manage stock sustainability, several countries have established minimum landing sizes. The species' resilience is attributed to its abundance and broad distribution, yet ongoing conservation efforts remain essential to preserving its population (Collette et al., 2011; ICES, 2019).

2.2 Composition of Titus (*Scomber Scombrus*)

S/N	Component	Value	Unit
1.	Moisture	63-75	%
2.	Protein	18-20	%
3.	Fat	10-20	%
4.	Ash	1.2-1.5	%
5.	Energy	205-300	Kcal/100g
6.	Omega-3 Fatty Acids	1.5-2.0	g/100g
7.	Vitamin A	40-50	IU
8.	Vitamin D	10-20	IU
9.	Vitamin E	0.5-1.0	mg
10.	Calcium (Ca)	10-20	mg
11.	Iron (Fe)	1-2	mg
12.	Magnesium (Mg)	30-40	mg
13.	Phosphorus (P)	150-250	mg
14.	Potassium (K)	250-350	mg
15.	Sodium (Na)	60-100	mg
16.	Zinc (Zn)	0.5-1.0	mg
17.	Selenium (Se)	35-50	µg

2.3 Lipid Oxidation

Oxidative rancidity leads to the qualitative deterioration of seafood and other muscle food, resulting in quality loss and shortening shelf life. The oxidation process of unsaturated fatty acids or triglycerides in marine food initiates the formation of free radicals and hydroperoxides. Subsequently, hydroperoxides undergo polymerization, forming dark-colored organic polymers. Additionally, various compounds such as ketones, aldehydes, alcohols, hydrocarbons, acids, and epoxides may be generated (Cheng, 2016).

Lipid oxidation occurs in both fresh and frozen marine food and can be catalyzed by metal ions. Oxidized unsaturated lipids have an affinity for proteins, leading to the formation of insoluble lipid-protein complexes. This phenomenon contributes to the undesirable characteristics of poorly stored marine food, including poor flavor, toughened texture, and unappealing odor. Thus, controlling oxidation has become increasingly important in food preservation. Antioxidants are among the most effective inhibitors of lipid oxidation, serving as agents to mitigate the oxidative process and extend the shelf life of seafood products (Azhar et al., 2006).

2.4 Cured and Uncured Surf

Cured seafood refers to fish and shellfish that have been preserved through methods such as salting, smoking, drying, or fermenting. These processes inhibit bacterial growth, extend shelf life, and often impart distinct flavors. Examples of cured seafood include smoked salmon, salted cod, dried shrimp, and pickled herring. In contrast, uncured seafood is fresh or frozen fish and shellfish that have not undergone any preservation processes beyond basic refrigeration or freezing, maintaining their natural moisture content and flavors. Examples of uncured seafood include fresh tuna, raw oysters, and frozen shrimp (Feng et al., 2016).

The lipid content in seafood varies significantly depending on the type of fish or shellfish and whether it is cured or uncured. In cured seafood, the lipid content can be affected by the curing process, primarily through dehydration, which concentrates the remaining lipids. However, the overall lipid composition may not significantly change unless the curing process involves the addition of fat, such as in oil-cured products. For instance, smoked salmon contains approximately 10-15% lipids, depending on the salmon species and smoking method, while salted cod, a lean fish, typically has around 1% lipid content as the salting process does not add significant lipids (Sirot et al., 2008).

Uncured seafood retains its natural lipid content, which can vary widely among different species and even within the same species based on their diet, habitat, and season. Fresh tuna, for example, shows a considerable range in lipid content: Bluefin tuna has about 5-15% lipids, whereas Yellowfin tuna has about 2-5%. Raw oysters are generally low in fat, around 2-3%, and frozen shrimp typically contains about 1-2% lipids. Understanding these differences in lipid content helps in selecting seafood based on dietary preferences and nutritional needs (Feng et al., 2016).

2.5 Hydroxyl Radical Scavenging Activity Studies of Fish Oil

Today, fish oil enriched with omega-3 is in high demand due to its well-established health benefits. Various fish by-products, including heads, tails, backbones, and viscera, are rich sources of these essential fatty acids, crucial for human health (Carvajal et al., 2014). Omega-3s, being polyunsaturated fats, cannot be synthesized by the body, emphasizing their importance in the diet (Savitha et al., 2014).

Studies have delved into fish oil's potential as an antioxidant, particularly in scavenging hydroxyl radicals implicated in oxidative stress. Fish oil, abundant in omega-3 fatty acids like EPA and DHA, has shown efficacy in neutralizing these damaging radicals. Although omega-3s aren't deemed super antioxidants, they play a significant role in mitigating oxidative stress, crucial in combating chronic diseases such as cancer, rheumatoid arthritis, and Parkinson's disease (Catherine, 2015).

The antioxidant activity of fish oil is largely attributed to the presence of omega-3 fatty acids like EPA and DHA, which possess multiple double bonds in their structure. These bonds enable them to act as antioxidants, counteracting the harmful effects of oxidative stress. Additionally, studies have explored synergistic effects between fish oil and other antioxidants like vitamin E and astaxanthin, enhancing the overall antioxidant capacity of fish oil (Airanthi et al., 2011).

Antioxidants, including omega-3 fatty acids, serve as protective shields against oxidative stress by neutralizing free radicals. DHA and EPA, abundant in fish, offer myriad health benefits, from shielding against heart diseases and strokes to reducing triglycerides, blood pressure, and inflammation, thereby mitigating plaque formation (Catherine, 2015). These effects render omega-3s invaluable in managing conditions like Crohn's disease, rheumatoid arthritis, and asthma.

Furthermore, omega-3s derived from fatty fish play crucial roles in heart protection and brain function maintenance. Sarah Rafat, RD, emphasizes their anti-inflammatory properties, particularly significant in cancer contexts. Omega-3s aid in curbing tumor growth and enhancing chemotherapy tolerance by promoting cancer cell self-destruction (Peter, 2012).

In conclusion, fish oil, rich in omega-3 fatty acids, exhibits potent antioxidant properties, offering protection against oxidative stress and associated diseases. The synergistic effects of fish oil with other antioxidants further enhance its antioxidative capacity, making it a valuable component in promoting overall health and well-being (Airanthi et al., 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

Beakers, Dissecting Kit, Micropipettes, Mortar and Pestle, Pipettes, Sample Bottles, Test Tubes, Reagent Bottles, Ascorbic Acid, Chloroform, Distilled Water, DMSO (Dimethyl Sulphoxide), EDTA (Ethyl Diamine Tetraacetic Acid), Iron EDTA; FAS (Ferrous Ammonium Sulphate), Methanol, NASH Reagent (Sodium Hydrosulfide), TCA (Tetrachloroacetate), Centrifuge, Spectrophotometer, Water Bath, Weighing Balance, Scubia (*Scubia Scubium*), Salt, Ginger, Garlic, Onions, Vegetable Oil.

3.2 METHODS

3.2.1 Sample Collection

The samples were obtained from Uselu Market in the Egor Local Government Area of Edo State, Nigeria. They were sent to the Department of Animal and Environmental Biology at the University of Benin for identification and verification of the specimen in which the batch ID is UNIBEN-AEB-SS-2024-001.

3.2.2 Preparation of Samples

The samples were cleaned to eliminate any potential contaminants, 5 sample was prepared. The samples was sub-divided into:

1. Raw Sample-RS
2. Raw Marinated Sample-RMS
3. Raw Boiled Sample-RBS
4. Raw and Fried Sample-RFS
5. Marinated and Boiled Sample-MBS
6. Marinated Boiled and Fried Sample-MBFS

1. **Raw Sample**- This sample was simply cut and kept in a sample bottle.
2. **Raw Marinated Sample**- This sample was marinated with a blended mixture of spices.
3. **Raw Boiled Sample**- This sample was boiled without any marination.
4. **Raw and Fried Sample**-This sample was fried without any marination
5. **Marinated and Boiled Sample**- This sample was marinated before boiling.
6. **Marinated Boiled and Fried Sample**- This sample was marinated with spices, boiled, and then fried.

3.2.3 Total Lipid Extraction

Each samples were prepared by measuring 5g of sample using a weighing balance and then blended/maturated to a paste, after which 5ml of distilled water, 5ml of methanol and 10ml of chloroform in the ratio of 1:1:2 are added. The homogenate was then centrifuged at 4000 (rpm) for 10 min. The lipid phase was then collected with the aid of a pipette and transferred to fresh test tubes and labelled accordingly.

3.3 4-HYDROXYL RADICAL SCAVENGING ACTIVITY ASSAY PROCEDURES

Principle

The hydroxyl radical scavenging activity assay hinges on generating hydroxyl radicals (•OH) through the Fenton reaction, where ferrous ions (Fe^{2+}) react with hydrogen peroxide (H_2O_2). The reaction proceeds as follows:

$$\mathbf{Fe^{3+} + \bullet O_2^- \rightarrow Fe^{2+} + O_2} \qquad (1)$$

$$\mathbf{Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO\bullet + OH^-} \qquad (2)$$

$$\mathbf{Fe^{3+} + HO^- \rightarrow Fe^{2+} + \bullet OH} \qquad (3)$$

Hydroxyl radicals are highly reactive and can cause oxidative damage to biological molecules, such as sugars, amino acids, lipids, and nucleotides (Halliwell & Gutteridge, 2015; Zeng et al., 2020). In this assay, deoxyribose reacts with hydroxyl radicals to form malonaldehyde (MDA), which then reacts with thiobarbituric acid (TBA) under heated, low pH conditions, producing a pink chromogen. The intensity of the pink color, measured at 532 nm, is directly proportional to the amount of MDA formed and thus to the hydroxyl radical activity(Owen et al., 2018).

Antioxidants present in the test sample (such as fish oil) can neutralize hydroxyl radicals, reducing MDA formation. This reduction is quantified by comparing the absorbance of the sample to a control without antioxidants, expressing the hydroxyl radical scavenging activity as the percentage inhibition of MDA formation. Given their high reactivity and potential to cause cell damage in vivo, hydroxyl radicals' scavenging is a crucial antioxidant activity, underscoring the importance of evaluating substances for their ability to mitigate oxidative stress(Bae et al., 2020; Watanabe et al., 2019).

Procedure

Iron EDTA solution 1ml, 0.5ml of EDTA solution, 1ml of DMSO and 0.5ml of ascorbic acid were added to the various test tubes properly labelled containing 1ml of the samples to carry out research on. The mixture was incubated in a boiling water bath at 90°C for 15minutes. After incubation, 1ml of Ice Cold TCA and 3ml of NASH reagent were added. The reaction mixture is then again incubated at the same temperature for the same period of time. Then allowed to cool, absorbance is then read at 412nM and values of the various samples are taken.

Calculation

The % hydroxyl radical scavenging activity is calculated by the following formula

$$\%\text{HRSA} = \frac{Abs\ Control\ - \text{Abs Sample}}{Abs\ Control} \times 100$$

Where %HRSA is the Hydroxyl Radical Scavenging Activity, Abs control is the absorbance of control and Abs sample is the absorbance of the extract.

Protocol

	SAMPLE	**BLANK**
Extract	1ml	
Iron EDTA	1ml	1ml
EDTA Solution	0.5ml	0.5ml
DMSO	1ml	1ml
Ascorbic Acid	0.5ml	0.5ml
	3ml + Sample Incubate	
Ice Cold TCA	1ml	
Nash Reagent	3ml	

3.4 Statistical Analysis

The data obtained were analyzed using the SPSS software, version 21. Student t-test was employed to compare the mean and standard error of mean and values of $P<0.05$ as significant.

CHAPTER FOUR

4.1 RESULTS

Table 4.1: The level of hydroxyl radicals of cured and uncured boiled and fried fish (*Scomber scombrus*) oil.

Group	**Level of hydroxyl radicals (%)**
RS	24.05 ± 8.02
RBS	44.05 ± 16.87
RCS	15.35 ± 7.33
CBS	30.27 ± 16.99

*Values are expressed as Mean ± SEM

Key: RS- Raw Sample, **RCS-** Raw Cured Sample, **RBS**- Raw Boiled Sample, **CBS-** Cured Boiled Sample.

Based on the table, the Raw Cured Sample (RCS) shows the highest scavenging activity with the lowest hydroxyl radical level (15.35 ± 7.33), followed by the Raw Sample (RS) with 24.05 ± 8.02. In contrast, the Cured Boiled Sample (CBS) shows a moderate increase in hydroxyl radical levels at 30.27 ± 16.99, indicating decreased scavenging activity compared to RCS and RS. The Raw Boiled Sample (RBS) exhibits the highest hydroxyl radical level (44.05 ± 16.87), showing a significant decrease in scavenging activity. Overall, curing significantly enhances antioxidant activity, while boiling tends to reduce it.

CHAPTER FIVE

5.1 DISCUSSION

The study shows clear trends in the scavenging activity of *Scomber scombrus* oil under different processing methods. The Raw Cured Sample (RCS) had the highest scavenging activity, evidenced by the lowest hydroxyl radical level (15.35 ± 7.33), indicating curing enhances antioxidant properties. In contrast, the Raw Boiled Sample (RBS) showed the highest hydroxyl radical levels (44.05 ± 16.87), suggesting that boiling significantly reduces antioxidant activity. The Cured Boiled Sample (CBS) exhibited moderate scavenging activity, showing that boiling after curing still diminishes antioxidant potential but less so than boiling alone. These patterns highlight the positive impact of curing and the negative effect of boiling on antioxidant capacity.

This aligns with prior research, which shows that curing preserves beneficial compounds in fish oils, enhancing their antioxidant properties (Carvajal et al., 2014). Conversely, boiling has been shown to cause oxidative degradation, reducing the oil's efficacy in scavenging free radicals (Feng et al., 2016). The findings suggest that cured *Scomber scombrus* oil could be a valuable natural antioxidant, potentially useful in reducing oxidative stress linked to chronic diseases like cardiovascular conditions. However, boiling should be minimized to retain these benefits.

Limitations of the study include a small sample size and a narrow focus on hydroxyl radicals. Future research should explore other radical types and the effect of alternative cooking methods like frying. Further studies should also assess the long-term stability of antioxidant compounds in different storage conditions. Overall, *Scomber scombrus* oil, particularly when cured, holds promise as a natural antioxidant, but more research is needed to optimize its processing and application.

CONCLUSION

The study highlights that curing enhances the antioxidant properties of scubia fish oil, with raw cured samples (RCS) showing the highest scavenging activity. Boiling and frying, however, diminish these properties. These findings suggest that minimally processed, cured scubia fish oil could serve as a potent antioxidant. Future research should focus on optimizing processing methods to retain antioxidant benefits and exploring other potential health benefits of scubia fish oil.

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