# EFFECTS OF SEASONAL VARIABILITY AND PROCESSING METHODS ON BIOCHEMICAL COMPOSITION OF SELECTED FISH FROM TANZANIA MARINE WATERS

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**A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY OF THE OPEN UNIVERSITY OF TANZANIA**

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## CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by The Open University of Tanzania, a thesis entitled: **Effects of Seasonal Variability and Processing Methods on Biochemical Composition of Selected Fish from Tanzania Marine Waters**, in fulfillment of the requirements for the degree of Doctor of Philosophy of The Open University of Tanzania.

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## DECLARATION

I, **Stella Mabondo Yonah Shija,** do hereby declare that this thesis is my own original work and that it has not been presented to any other University for a similar or any other degree award.

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Date

# DEDICATION

This thesis is dedicated to my late Father **Aaron Mabondo** and to the rest of my family including my lovely Mother **Elizabeth Mabondo**, Husband **Yonah Shija**, two Daughters (**Elizabeth** and **Esther**) and Son (**Eric**) for their patience and perseverance during the period of my research.

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## ABSTRACT

Mineral and proximate contents in selected fish species (*Alectis ciliaris*, *Lethrinus harak*, *Rastrelliger kanagurta* and *Siganus canaliculatus*) from Tanzania marine waters were assessed in changing seasons (wet and dry) and different processing methods (frying and boiling). The fish samples were randomly collected from four selected locations (Tanga, Bagamoyo, Dar es Salaam and Mtwara) and treated as appropriately. Whereas mineral (Potassium (K), Sodium (Na), Calcium (Ca) and Magnesium (Mg)) contents were analyzed by using Atomic Absorption Spectrophotometry (AAS) while proximate (ash, crude protein, lipid, moisture) contents were determined using Association of Official Analytical Chemists (AOAC) standard methods. Changing of seasons influenced mineral contents (except in Ca) in all the fish species. Significantly high K, Na and Mg contents were observed during dry season compared to wet season. Similarly, protein and lipid contents were high in wet season, while moisture and ash contents were high in dry season. The effect of changing seasons in proximate contents was significant ($p < 0.05$) except in ash. The K, Na and Mg contents in fish were decreasing by varying processing methods (raw > fried > boiled) except in some fish species where high contents were observed in boiled fish. Regardless of the processing method, Ca content was more or less the same in all the selected fish species. Whereas derived model accurately predicted variations of mineral and proximate contents due to changing of seasons, and accurate content predictions were only possible with potassium due to processing methods. Frying process had a significant effect on proximate contents in the fish species than boiling process except in ash. Derived model failed to predict variation of lipid, protein and moisture when using boiling processing method. However, boiling processing method

had a lower influence on proximate contents in fish compared to frying processing method. Further research is needed on factors that cause variations in Calcium mineral. In addition, more research is required on variations of mineral and proximate contents due to other processing methods such as steaming and microwaving.

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| | |
|---|---|
| AAS | Atomic Absorption Spectrophotometer |
| ANOVA | Analysis of Variance |
| AOAC | Association of Official Analytical Chemists |
| ARU | Ardhi University |
| DHA | Docosahexanoic Acid |
| DO | Dissolved Oxygen |
| EPA | Eicosapentaenoic acid |
| FAO | Food and Agriculture Organization |
| GDP | Gross Domestic Product |
| IBM - | Individual Based Modes |
| IO | Indian Ocean |
| NBS | National Bureau of Standards |
| NFE | Nitrogen free extract |
| NPN | Non-protein Nitrogen |
| OCGS | Office of the Chief Government Statistician |
| PC | Principal Component |
| PCA | Principal Component Analysis |
| PUFA | Polyunsaturated fatty acids |
| SPSS | Statistical Package for the Social Sciences |
| SUA | Sokoine University of Agriculture |
| UDSM | University of Dar es Salaam |
| WIO | West Indian Ocean |

# CHAPTER ONE

# GENERAL INTRODUCTION

## 1.1 General Background

Fish is a natural source of polyunsaturated fatty acids, dietary sources of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), a form of omega-3 that has great health benefits (Parker *et al.,* 2019). Fish are also highly proteinous as they contain essential amino acids which are the building materials of proteins (Ahmad *et al.,* 2018). Other biochemical components are minerals (Potassium, Calcium, Sodium, Magnesium and Phosphorus) and trace elements (Zinc, Manganese, Iron and many others). So, the edible portion of fish, which has water, protein, lipids (fat or oil) and ash (minerals) (Ahmad *et al.,* 2018) has to depict little or no variation in the biochemical composition (Njinkoue *et al.,* 2016).

The biochemical composition of fish may be altered by the fish feeds, salinity, geographical location, seasons and processing methods (Bogard *et al.,* 2015). Geographical location and seasonal changes do affect the fish environment due to the availability and composition of feeds, which consequently affect the chemical composition of their muscle fillet. The general composition of fish body is the final function of the available food fed by fish and the assimilative capacity of individual fish. Likewise, the fluctuation of water temperature across the seasons (wet and dry) and the activities of fish (reproduction and migration) could influence the biochemical composition of muscles (Bandarra *et al.,* 2001; Olsson *et al.,* 2003). There is a relationship between location and availability of fish on one hand and the climate and oceanographic conditions on the other (Van Der Elst *et al.*, 2005).

Fish can be eaten raw but preference is when treated under various processes such as boiling, grilling, frying and may other (Ersoy & Özeren, 2009). These processes tend to improve the flavor, taste, inactivate pathogenic microorganisms and increase shelf life (Bognár, 1998). This could lead to important biochemical changes in the fish composition (Weber *et al.,* 2008). The principal changes that occur during processing are oxidation during heating, which is catalyzed by heat, light and additional trace metals or enzymes that generate free radicals (Loughrill & Zand, 2016). Heat may also cause denaturation and mineral solubilization (Gladyshev *et al.,* 2007). The fish consumer consider the flesh texture, taste, protein and fat contents (Pal & Ghosh, 2013).

## 1.2 Statement of the Research Problem

Changes of the composition of fish due to seasonal variation and processing methods has raised concern to human health (Ozogul *et al.* 2011; Aberoumand & Ziaei-Nejad, 2015). The mineral and proximate contents of fish is directly related to the type of feed, which can vary with seasons (Khitouni *et al.*, 2014). Seasons influence the fish feed's quantity, contents and availability and thus cause changes in fish body composition.

Common processing methods in fish include among others frying, boiling, sun drying and smoking. These processing methods usually improve the hygienic quality of the fish (Abdulkarim *et al.* 2015) and inactivate the pathogenic microorganisms (Bognár, 1998). However, these processing methods can cause modification in the minerals, proximate and amino acids, thus change the biochemical composition of fish (Laly & Venketeswarlu, 2016).

Little information is available on the variation of biochemical contents (minerals and proximate components) in fish (*Alectis ciliaris*, *Lethrinus harak*, *Rastrelliger kanagurta*, and *Siganus canaliculatus*) from Tanzania Marine Waters due to changing seasons and after being subjected to different processing methods. Therefore, the aim of the study was to evaluate the effect of variation of seasons (wet and dry) and processing methods (frying and boiling) on the mineral and proximate contents of the selected fish. Predictive models were also employed to predict the effect of varying seasons and processing methods on mineral and proximate contents of fish.

## 1.3 Research objectives and Hypotheses

### 1.3.1 General research objective

The general objective of this study was to investigate the effects of seasonal variation and processing methods on the biochemical composition of selected fish from Tanzania marine waters.

### 1.3.2 Specific objectives

In order to address the main objective, the following specific objectives were formulated:

(i) To determine mineral contents (Potassium, Sodium, Calcium and Magnesium) of selected fish species from Tanzania marine waters at different seasons.

(ii) To determine proximate composition (ash, crude protein, lipid and moisture) of the selected fish species from Tanzania marine waters at different seasons.

(iii) To compare mineral contents of the selected fish subjected to different processing methods (frying and boiling).

(iv) To compare proximate composition of the selected fish subjected to different processing methods (frying and boiling).

(v) To derive a model that can predict changes in mineral and proximate contents due to seasonal variation.

(vi) To derive a model that can predict changes in mineral and proximate contents due processing methods.

### 1.3.3 Research Hypotheses

(i) There is no significant variation of the mineral contents of selected fish species due to varying seasons.

(ii) There is no significant variation of the proximate composition of selected fish species due to varying seasons.

(iii) There is no significant difference of mineral contents between raw and the processed fish.

(iv) There is no significant difference of proximate composition between raw and the processed fish.

(v) The derived model can accurately predict changes in minerals and proximate contents due to seasonal variation.

(vi) The derived model can accurately predict changes in minerals and proximate contents due to varying processing methods.

### 1.4 Significance of the study

The findings from this study may be used to raise awareness on the nutritional quality of the commonly available fish species. Recommendation on the appropriate fishing season and processing methods may be useful in getting the best nutritional utility of the consumed fish species. The biochemical data generated on the fishes may be useful to consumers interested not only in whether the fish tastes good, but also in its nutritional value, hence may help consumers in developing suitable processing method.

This study will render a platform to establish the amount of mineral and proximate composition available within the selected fish species under seasonal variation introduced to different processing methods. The knowledge generated from this study on fish nutrient contents and the factors affecting them may allow consumers, policy makers, pharmaceutical industries and hospitals to assess the health of fish itself and the quality of the nutrients that will be available to the consumers. The quality of fish itself that is very important for nutritional assessment and the derived models will be documented and made available to the consumers, scientists and researchers. Lastly, the study will provide a baseline for further studies on other unstudied fish species.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Diversity of marine fishes in West Indian Coast

East African coastline falls within Tropical Western Indian Ocean covering the coast of Somalia, Kenya, Tanzania, Mozambique and South Africa. The Western Indian Ocean has a large variety of habitats and oceanographic conditions giving it richness of marine species (Van Der Elst *et al.,* 2005). It contains many species although some fish and shrimps need more than one habitat for feeding, spawning and/or refuge.

There are methods of classifying fishes based on their migration exhibited (Myers, 1949). These are:

(i) Diadromous fishes migrate between sea and freshwater. They are divided into two groups a) Anadromous fish that breed in fresh water then spend most of their time in the sea e.g. *Salmo salar*, sea-lamphreys. b) Catadromous fishes that breed in the sea and spend most of their time in the freshwater e.g. *Anguilla*, some *Galaxias*.

(ii) Amphidromous fishes are those that migrate both ways to sea and freshwater not for breeding process E.g. some species in the families of Mugilidae.

(iii) Potamodromous fishes are those that live and migrate wholly in fresh water e.g. *Salminus labeo.*

(iv) Oceanodromous fishes are those which migrate and live entirely in the sea e.g. tunas, herrings.

The coast of East Africa contains a number of essential and key natural resources, forests and a variety of ecosystems supporting the rich biodiversity of fish. The fish families found in the area include the Lethrinidae, Lutjanidae, Siganidae, Scaridae, Labridae, and Mullidae (Wantiez *et al.*, 1997).

## 2.2 Importance of Marine fishes

Marine products provides substantial amounts of different beneficial nutrients such as nutritional and digestible proteins, lipid, essential minerals and highly unsaturated fatty acids (Phillips *et al.*, 2015). The medicinal and perceived nutritional values with the increase of population has made the marine products market demand to increase. Fish and fishery products are among the marine products that have a high nutritional quality and thus play an important role in the human diet. The researchers Erkan & Özden (2007) mentioned the mineral components found in fishery products such as potassium, magnesium, calcium, iodine, phosphorus that are important for human nutrition. Fish may be heavy but the nutrients are contained in the edible portion which is the fillet (Lilly *et al.*, 2017). It was reported that fish body composition have high protein content (18 % - 22 %), low unsaturated fat less than 3 %, moisture ranged from 74 % - 90 % and ash was 1.01 % - 1.50 % (Shehawy *et al.*, 2016).

Fishing plays an important role as a source of protein-rich food and employment (Francis & Bryceson, 2001). Despite of this, marine fish have other benefits such as fish scales that are used for making artificial pearls, decorative beads and glue production using raw materials from the air bladder. The fish silages are used as supplements in animal feed, the fish oil from the liver are used as a source of Vitamin D and nitrogenous fertilizers from fish surplus (Ytrestøyl *et al.*, 2015). In the past it was reported by Kamukuru *et al.* (2005) that, fish such as snapper were an important commercial reef-fish in Tanzania and in Mafia Island the snapper contributed 41.6 %. to the total marine fish catches (Kamukuru *et al.,* 2005). The snappers were also heavily targeted species in Fiji (Golden *et al.,* 2014).

Fish proteins have high biological values because they are characterized by the presence of essential amino acids in good proportions (Rahman *et al.,* 2018). According to Mc Dougall (2002) essential amino acid composition was one of the most important nutritional qualities of protein. The authors Salihu-Lasisi *et al.* (2013) added that, the fish protein was better than beans plant with greater satiety effect than other sources of animal protein like chicken and beef (Mohanty *et al.,* 2016). Its amino acid helps in repair of cells and the iodine found in fish prevents goiter or regulates the body's processes (Franceschi, 1998). In most of the fish species, the protein amount in its fish muscles is between 15 and 20 per cent (Craig & Helfrich, 2002). Fish consumption in human diet during sickness and convalescence have good nutritional value (Rani *et al.,* 2016).

Concomitantly, promotion of fish consumption as health food and brain food has become a global concern (Bagthasingh *et al.,* 2016). It was known that fish provides employment of about 800 million people globally (Mohanty *et al.,* 2016). Under this scenario, fisheries is one of the fastest growing food production sectors and an alternative source for providing nutritional content and food security to people (Mohanty & Ayyappan, 2017). In human health, minerals components such as potassium, magnesium, calcium, iodine phosphorus and many other are important in human nutrition (Erkan & Özden, 2007). Sodium and potassium work together in the transmission of nerve impulses and keeping electrolyte balance; sodium and chlorine maintain osmotic pressure. Zinc is mostly found as a cofactor in enzyme reactions, iron forms part of the haemoglobin molecule which transport oxygen around the body (Alaş *et al.,* 2014). Other functions of minerals in human health are proper muscle's functioning, heart's health and may prevent diabetes. It was believed by the researchers Lilly *et al.* (2017) that the deficiency in these principal nutritional mineral elements induces a lot of malfunctioning including reduced productivity, inability of blood to clot, osteoporosis, anemia and others.

There are several human health benefits in fish consumption that was strongly recommended as reported by Þorgilsson & Gunnlaugsdóttir (2010). This was justified due to the presence of the long chain polyunsaturated fatty acids (PUFA) omega 3 (Bannenberg *et al.*, 2017). The essential fatty acids and minerals in fish are beneficial to retina, nerves, growth, brain functioning and development including nutrients for growth and development of the human body (Leng *et al.*1999; Penido & Alon, 2012, Claxton, 2016). Chronic diseases such as coronary heart disease can be prevented by consuming at least two meals of fish, preferably oily fish, per week (Harris *et al.,* 2004). It was reported that eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) in fish oil helps in bone formation and metabolism, also in the prevention of cardiovascular disease, especially for foetus, neurodevelopment in infants, adolescents, pregnant and lactating women (Muskiet *et al.,* 2006). The growth of the cells found in human brain in the mothers' womb to a toddlers depends on the consumption level of omega-3 and its derivatives that are EPA and DHA (Malan, 2014).

Other importance of long-chain *n*-3 PUFA is the curative and preventive effects on coronary artery diseases, cancer, fat glycemic control, rheumatoid arthritis, multiple sclerosis, psoriasis and inflammation (Yesilayer & Genc, 2013). In Inuit and Japan where there is high consumption of fish, they have lower rates of acute myocardial infarction, other ischemic heart diseases and atherosclerosis (Iso *et al.*, 2006).

Minerals such as calcium and phosphorus are responsible in prevention of rickets and osteomalacia for growing children (Carpenter *et al.*, 2011). They also help in reduced bone growth, bone volume and increase skeletal fragility (Carpenter *et al.*, 1992). Others importance of minerals are enzyme-cofactors, maintenance of colloidal system, regulation of acid-base equilibrium (Chanda *et al.* 2015) and essentially for normal growth (Prasad, 1991).

## 2.3 Biology of the selected fish species

Vertebrates in phylum Chordata and subphylum Vertebrata are animals with a backbone or spinal column. Fish or Pisces is one of the classes in Chordata Phylum containing six (6) sub classes being Actinopterygii, Sarcopterygii, Holocephali, Elasmobrachii, Cephalaspidomorphi and Myxini. The selected fish species from the sub class Actinopterygii, also known as ray-finned fishes as their fins being supported by rays, are the major portion of living vertebrate diversity (Faircloth *et al.*, 2013). It consists of 42 orders 480 families (Stanley & Lynne, 2018) being the largest group of vertebrates with more than 32,000 species (Sallan, 2014). Most of the ray-finned fish have a swim bladder which is a unique feature that enables them to maintain buoyancy during swimming as they move up and down. The habitat of Actinopterygians are all aquatic environment such as abyssal plane, montane river and intertidal coastline where they feed on micro-plankton to mammals (Nelson, 2006). The different biology of fish species may cause variation of nutrient composition as reported by (Bogard *et al.*, 2015). According to Solanki *et al.* (1976) the nutrient contents of middle and tail portions of the fish body are almost the same. The following are the biology of the selected fish species in the study; *Alectis ciliaris*, *Rastrelliger kanagurta*, *Lethrinus harak* and *Siganus canaliculatus*

### 2.3.1 Biology of *Alectis ciliaris*

Figure 2.1 African pompano (*A. ciliaris*) Source: Author, 2017

Among the fish species in Actinopterygii, Pompano that is under the family Carangidae and the Order Perciformes is one of the most highly desired marine fishes. The researchers Chavez *et al.* (2011)reported that pompano was recognized as a premium fish popular in high-end restaurants. The *A. ciliaris* is commonly known as

African pompano with local name *Kolekole*. The *A. ciliaris* is pelagic, very active with a compressed, deep body that lengthens with age so that the depth decreases from 88 % to 38 % of body length (Lieske & Myers, 1994).

Their color is bluish metallic touch dorsally and the opening at the upper end of the gill has a dark spot (Lieske & Myers, 1994). Juvenile African pompano have long trailing filaments that come off their dorsal and anal fins and disappears when older. They live on sandy and stony bottoms of water at a depth of not more than 60m and temperatures between 18-27$^{o}$C (Lieske & Myers, 1994). At adult stage, *A. ciliaris* are solitary feeding on sand mollusks and other hard-shelled invertebrates (Lieske & Myers, 1994) near coral/barrier reefs and close to ship wreckage or debris. A study done in India discovered that the reproduction of fish larvae is usually collected from March to May (Board, 2016).

### 2.3.2 Biology of *Lethrinus harak*

The *L. harak* which belongs to the family Lethrinidae are also known as Snappers with a local name *Changu.* They are a prominent family of predatory fishes found in all tropical waters and often associated with reef or mangrove habitats (Unsworth *et al.*, 2009). Mackerel are usually found solitary or in small schools over shallow sandy, lagoons, coral rubble, mangroves, channels and seagrass areas inshore and near coral reefs. They feed on polychaetes, crustaceans, small fish, mollusks and echinoderms (Carpenter & Allen, 1989). *L. harak* remains one of the few numerically abundant, carnivorous fishes encountered on reef flats (Taylor *et al.*, 2012).

Figure 2.2 Snappers (*L. harak*) Source: Author, 2017

### 2.3.3 Biology of *Rastrelliger kanagurta*

The fish species *R. kanagurta,* which belongs to family Scombridae is commonly known as Indian Mackerel with a local name *Kibua*. They are a very homogeneous group being carnivorous fishes feeding on plankton, crustaceans, mollusks, fish eggs, and small fish (Stanley & Lynne, 2018). The body of the mackerel is rounded and torpedo-shaped with slender keeled tail base, a forked tail. They have a velvety skin with a row of small finlets behind the dorsal and anal fins (Stanley & Lynne, 2018).

Figure 2.3 Mackerel (*R. kanagurta*) Source: Author, 2017

Mackerel's egg is average 1 mm (0.04 inch) in diameter and buoyant (Stanley & Lynne, 2018). The fish adults being oceanodromous occur in coastal bays, harbors and deep lagoons, usually in some turbid plankton-rich waters (Stanley & Lynne, 2018). They are predaceous, swift swimmers and powerfully muscled swimming actively in the upper 25-30 fathoms during warmer seasons and deep as 100 fathoms in cold season (Stanley & Lynne, 2018).

### 2.3.4 Biology of *Siganus canaliculatus*

*Siganus canaliculatus* under the family Siganidae, locally called *Tasi* is also known as rabbit fish due to its similarity of the rabbit nose. Their maximum length is 30cm (Vidthayanon, 1997) with a body silvery gray above, silvery below; a touch of olive green on nape and upper surface of head; fright pattern mottled with pale cream and dark brown (Lukacs & Bhadra, 2012). The life span of the species is estimated to be

7.8 years (Riede, 2004). The fish species are found in shallow and coastal waters of at least 40m depth (Jaikumar *et al.* 2011) in brackish water, reef-associated and oceanodromous (Riede, 2004). According to the Carpenter *et al.* (1997) the fish species were found in rocky, sandy seaweed bottoms hard-bottom structures and around the mouths of rivers in turbulent waters (Carpenter *et al.*, 1997). The fish species are locally very plentiful with their juveniles occurring in very large schools. The fish species are herbivorous in a small family of algaevorous (Carpenter *et al.* 1997) feeding on scraping benthic algae from rocks, corals and to a lesser extent on seagrasses. Taylor *et al.* (2012) recorded that between April and July, spawning was observed, but gonadosomatic indices suggest a second less defined spawning event in November.

Figure 2.4 Rabbit fish (*S. canaliculatus*) Source: Author, 2017

### 2.4 Effects of Seasonal Variation on Mineral Composition of Marine fish

The potential elements for fish body's functions are calcium, phosphorus, sodium, molybdenum, chlorine, magnesium, iron, selenium, iodine, manganese, copper, cobalt, zinc and many others. Seasons affect the chemical composition of fish and its nutritional quality (Khitouni *et al.,* 2014). The feeding habit and climatogical differences between two seasons may affect the biochemical composition of the fish species (Balogun & Talabi, 1986). In addition, feeding of adult fishes range from sieving phytoplankton or grazing algae, to suction feeding on benthic invertebrates and to devouring other fishes whole or in portions (Bone & Moore, 2004). This may be due to climatological conditions that affects the fish body chemical composition at different geographical locations (Balogun & Talabi, 1986). A study done by Khawaja & Jafri (1968) reported that, there was a direct correlation of seasonal variations in calcium and phosphorus seen in young *O. punctatus* showing a bimodal cycle. High values occurred during pre- and post-monsoon months and low values during winter and monsoon months which is related to the feeding intensity and growth of the fish. However, Ali *et al.* (2013) from Oman concluded that seasonal variability did not affect the mineral composition of fish.

Observation were made by Khitouni *et al.* (2014) when investigating the mineral contents in *Diplodus annularis* collected from Tunisia Coastal water. The value of the mineral content in the muscles of *D. annularis* was found as follow: Ca > K > Na > Mg > Fe > Zn, whereas Potassium and Calcium were found in all seasons. The research indicated that, there is significant difference in mineral contents of different fish (Barua *et al.* 2012; Kasozi *et al.,* 2014). Similar research was done in Lake Victoria fish by Abdulkarim *et al.* (2015) where *L. niloticus* and *O. niloticus* had higher Calcium, Potassium, Phosphorus, Sodium and Magnesium content in wet season. The researcher suggested that, higher mineral content in fish samples during heavy rainfall was probably caused by more nutrients carried in the runoffs to the natural habitat, rivers and streams, lake and the ocean, thus exposed to the fish. These results are in contrary to those reported by Saoud *et al.* (2008) who stated that, in wet season natural productivity decreased as some nutrient reserves may decrease.

It was also noted that high content of ash during dry season is responsible with more mineral concentration than wet season as reported by Adelakun *et al.* (2017) who worked with Catfish (*Clarias gariepinus*). The results showed that, potassium had higher content in dry season and slightly lower in wet season. Sodium content were maintained in both dry and wet season, while calcium content was lower in dry than wet season. According to Olgunoglu *et al.* (2014) where the results on Mesopotamian Catfish (*Silurus triostegus*) collected from Atatürk Dam Lake of Turkey agreed with the results of the Adelakun *et al.,* (2017). It was reported that, in summer (dry season), mineral contents were higher than during winter (wet season). During winter, minerals content were 80.21 ± 0.55 g/100g calcium, 179.20 ± 0.20 g/100g magnesium, 1281.36 ± 33.63 g/100g phosphorus and the highest content was seen in potassium 2171.67 ± 4.72 g/100g. In summer, highest content was seen in potassium with 3132.67 ± 2.51 g/100g, phosphorus 1557.25 ± 31.68 g/100g, magnesium 265.23 ± 1.07 g/100g while lowest content 103.56 ± 0.06 g/100g was seen in calcium.

Seasonal variation influences the composition of fish feeds and cause changes in amount and quality of the fish feeds. The opinion was in agreement with a study done in Koi species *Anabas testudineus*, from India experimented at different seasons (Paul *et al.*, 2015). Potassium and sodium contents were found to be higher in winter (dry season) and lower in summer (wet season). Likewise, calcium content was high during summer (wet season) and dropped in winter (dry season).

The different mineral contents between species may be a result of their diverse ecological needs, metabolism, feeding habits, and habitats. Other reasons of the differences in mineral contents are fish feeds, metabolic rate and mobility of the fish related to geographical and seasonal factors. The absorption is also done through gills and skin from the surrounding water (Rombough, 1998). The ability of the fish to absorb the nutrients from the fish feeds in water causes variation in mineral concentrations (Fregene & Olanusi, 2012; Nurnadia *et al.*, 2013).

### 2.5 Effects of Seasonal Variation on Proximate Composition of Marine Fish

The fish proximate components are protein, lipids, moisture ash and other important constituents although available in small amount. The carbohydrates and non-protein compounds such as fiber sarcoplasm, free amino-acids and others that are generally ignored (Cui & Wootton, 1988).

Various fish species may not provide same nutrient value (Kozo *et al.* 1999) as they vary with seasons (Saoud *et al.*, 2008). Seasonal variations in relation to environmental factors such as temperature and salinity affect the chemical and nutritional composition of marine fish (Olsson *et al.* 2003; Nisa & Asadullah, 2011). Fish have the ability to absorb nutrients not only from their diets but also from water (Lall & Tibbetts, 2009). The exchange of ions from the aquatic environment across gills and skin of fish complicates the determination of the quantitative dietary requirements of minerals (Roy & Lall, 2006). The variation of minerals and proximate contents in tissues of marine and fresh water fishes at different locations and seasons has also been determined (Abdullahi, 2005, Olgunoglu *et al.* 2014, Abdulkarim *et al.*, 2015). They also influence the composition of their food as well as activities (reproduction and migration) including age, sex and size of fish (Gall *et al.*,1983). It was confirmed that, crude protein, lipid and fats composition varied with seasons (Bandarra *et al.* 2001; Celik, 2008; Nisa & Asadullah 2011).

The fish of same species may have differences in their composition and between individuals. It may be caused by sexual cycle, starvation periods for natural or physiological reasons (spawning or migration) (Khitouni *et al*. 2014), water quality and capture condition or due to shortage of food (Boran & Karacam, 2011; Bannenberg *et al.*, 2017).

Other effects on nutritional value may be caused by level of maturity, ecosystem, time and region of sampling. Fatty acids in fish species depends on environmental factors such as salinity, species habitat and temperature. Climatological differences as seasons stated by Abdulkarim *et al.* (2015) may affect the nutritional composition in fish. Other factors such as geographical location and genetic factors as lifestyle stage and size may also have an impact on the fish fatty acids (Yildiz, 2008, Ozogul *et al.*, 2011).

It was reported that cultures and geographical differences, count on the fish quality (Rasmussen, 2001). This was demonstrated by Alemu *et al.* (2013) whereby female *Oreochromis niloticus* had higher contents of protein and fats likewise fats and moisture concentration was higher in older fish than young while protein and ash was lower contents in older fish. The differences of the fish species affects fish nutrient profile (Kozo *et al.*, 1999). This was experimented by Njinkoue *et al.* (2016) when comparing two species *Pseudotolithus typus* and *Pseudotolithus elongates.* The values of the former and latter fish species were as follows: moisture 76.17 % and 78.24 %, fats 0.46 % and 0.36 %, protein 16.17 % and 13.4 %, ash 0.19 % and 0.83 % respectively.

It was discovered that 16 % of protein in the fish species was maintained while lipid levels varied seasonally with the moisture content (Leu *et al.,* 1981). In Nigeria, Effiong & Fakunle (2011) determined the proximate contents of different fish species and found the following; crude protein (21.62 - 60.57) %, ether extract (3.88 - 9.1) %, ash (1.35 - 5.88) %, moisture (8.80 - 17.16) % and dry matter (85.53 - 91.24) %. A report on ten (10) different commonly consumed fish species in Oman indicated that, moisture contents ranged from (67.0 - 78.8) %, ash (1.1 - 1.5) % and crude protein (19.1 - 26.1) % (Ali *et al.*, 2013).

Ashwini *et al.* (2016) from India found the variations of proximate constituents between sexes were as follows: male *Decapterus russelli* had protein (18.83 - 20.02) % female (18.85 - 19.91) %, ash in males (0.12 - 0.34) % females (0.13 - 0.34) %, fats in males (0.75 – 2.0) % females (0.78 - 1.99) % and moisture in males (76.23 -78.93) % females (76.63 - 78.02) %. A recent research done by Gul *et al.*, (2017) analyzed species from Pakistan that had moisture content ranging from (59.73 - 72.13) %, fats (1.0 - 6.0) %, ash (3.33 - 8.0) % and protein (15.33 - 22.67) %. This indicates that there are variation of proximate contents in different fish species.

### 2.5.1 Seasonal Variation on Crude Protein in Fish

Protein is an important constituent of foods that is used to evaluate the quality of the food as a major source of energy. It replaces the metabolic losses and damaged tissues and also contains the essential amino acids that are necessary for human health. The report of Steffens *et al.* (2006) stated that, the largest quantity of dry matter in fish was protein. In Karachi-Pakistan, Nisa & Asadullah, (2011) studied on mackerel and recorded a maximum protein content seen in June during rainy season while minimum content during dry season in December was used for metabolic energy. The crude protein values' record of mackerel species came into agreement with Bandarra *et al.* (2001) and to some extent with the findings of Celik (2008). The concept was also supported by the scientists Sahin *et al.,* (2011).

During a period of long starvation, the protein in the body of fish is used to cope with the condition (Yesilayer & Genc, 2013). The same findings were obtained by Kiran *et al.* (2017) in Andhra Pradesh in India where a decrease of protein contents were seen in rainy season. These results are in agreement to those obtained by Olgunoglu *et al.* (2014) who worked with Mesopotamian Catfish (*Silurus triostegus*) from Atatürk Dam Lake of Turkey. It was reported that, in summer (dry season), protein content was higher than in winter (wet season). These results were in agreement with those reported by Boran & Karacam (2011) in horse mackerel where in rainy season, lower protein contents were noted. A document of Güner *et al.* (1999) reported contrary results that there was a rising of protein and fat content in Indian Mackerel occurs during rainy season (November and December) in Black Sea was due to highest concentration of plankton. Between January and March which was early spring season, protein content was lower that was related to egg development known to be spawning period.

A study in *Neolissochilus hexagonolepis* done by Jyrwa & Bhuyan, (2016) from India remarked a higher concentration of protein during summer (wet) than in winter (dry) seasons. Similar results were obtained in Lebanon (Saoud *et al.* 2008) on eastern Mediterranean Sea where White Sea bream *Diplodus sargus* was experimented its proximate contents with seasonal variation. Protein contents were higher in winter season than summer.

Nurjanah *et al.* (2016) documented that, the composition of fish species may vary with size, where they are inversely proportional to each other. It was therefore concluded that, the smaller the size of the fish, the higher the moisture content and the smaller the protein content. It was also recorded that, these protein compounds contained in a water-containing materials are chemically bound to the constitution (Nurjanah *et al.*, 2016).

The concentration of protein (22.42 %) was found higher in the study done in shad fish by Güner *et al.* (1999), than those who reported 15.91 % by Boran & Karacam (2011). Variability might be caused by inability to compare the average values of six months with the values obtained from single sample collected from different fishing seasons.

### 2.5.2 Seasonal Variation on Lipids in Fish

Fishery products are high in essential minerals, polyunsaturated fatty acids (PUFAs), n-3 and n-6 and low in cholesterol content (Venugopal & Shahidi, 1996; Fallah *et al.*,2011). Nutritional quality of fish is largely associated with the content of essential fatty acids. Fish lipids are nutritionally rich due to the long-chain omega *n*-3 PUFA, especially eicosapentaenoic acid ((EPA) 20:5*n*-3) and docosahexaenoic acid ((DHA) 22:6*n*-3) (Harris *et al.,* 2004). The long-chain *n*-3 PUFAs are mostly obtained through the diet, since they cannot be synthesized readily by living organisms (Yesilayer & Genc, 2013). The knowledge of fish lipid is necessary to prevent oxidative or hydrolytic factors that would affect the fish quality (Deka *et al.,* 2012).

Despite of the difference of species habitat, other factors affecting fatty acids are the pattern type, amount of feed and the content of fatty acid the fish contains. The natural diet of the fish such as herbivorous, omnivorous or carnivorous (Sargent *et al.* 1995) geographical location and seasonal variation may also cause differences in fatty acids. This context was supported by the authors Saoud *et al.* (2008) that herbivore fish loses more reserves than carnivores. According to Hussain *et al.* (2016), herbivores and omnivorous fish showed a higher concentration of lipid during rainy season in summer while the findings on carnivorous fish were in contrast. The variation of lipid contents may be caused by the different types of feeds as main component of their diet.

The habitat has an effect on fat components in fish (Deka *et al.*, 2012). The content of lipid may be different depending of the fish species (Van Pelt *et al.,* 1997). The inactive fishes or those living at the sea floor, store the lipids in liver (Castell *et al.*, 1972). Seasonal variation may affect the chemical composition of fish, which may be as a result of different metabolic activities of the muscles. The lipid content oil sardine (*Sardinella longiceps*) muscles from Turkey, was low in June-July at 3-4 % during summer and increases in winter in November-December to 18 % (Guler *et al.*, 2007). Similar results were observed in a research done by Olgunoglu *et al.,* (2014).

In Jebba Basin, Nigeria Adelakun *et al.* (2017) during summer (rainy season) crude fat content in Catfish (*Clarias gariepinus*) was high (4.09 ± 0.03 %) and winter (dry season), the contents were slightly low (4.03 ± 0.16 %). It was discovered that, lipid content was inversely proportional to the moisture content. This is reflected by a decrease of moisture in the study of Bagthasingh *et al.* (2016) and fat content increases due to the heavy feeding during the period. This was proved by Boran & Karacam (2011) who determined fats in horse mackerel (*Trachurus trachurus*), shad (*Alosa fallax*), garship (*Belone belone)* and golden mullet (*Mugil auratus*) from Turkey. During autumn in October horse mackerel, shad, garship and golden mullet showed percentage (%) of fat content 8.42 ± 0.12, 9.34 ± 0.29, 4.95 ± 0.00, 4.81 ± 0.18 respectively. During rainfall in December, the results from the same species was elevated at 13.26 ± 0.20, 23.23 ± 0.09, 5.70 ± 0.03 except 2.62 ± 0.20 respectively.

The researchers Ünlüsay *et al.* (2010) reported that high level of fats in Indian mackerel during wet season in November and December reflected a high concentration of plankton in Black Sea. Similar results were observed in horse mackerel whereby in rainy season, higher fat were noted (Bandarra *et al.*, 2001; Boran & Karacam, 2011). These results disagreed with those noted by Kaçar *et al.* (2016) in *Silurus triostegus* from Turkey where the lipid content was high during spring and also during autumn but lower in winter (wet season).

It was earlier researched by Borges (1991) that, the spawning season of mackerel takes place during the first semester of the year that may be the cause of low fat content. The reason raised by Boran & Karacam (2011) on high fat contents was the preparation of spawning in shad that was indicated by heavy feeding causing a raise in fat content. It was known that, spawning period may influence the content of lipid in fish (Ozogul *et al.,* 2011). Salihu-Lasisi *et al.* (2013) mentioned that fish in cold water have high PUFA concentrations.

Great variations of lipid content is mainly caused by season and sexual maturity, in which there was high content during feeding season while decreasing in spawning (Chrisolite *et al.,* 2015). Likewise, the oily fish has a great difference in lipid composition especially in seasonal variation (Nazeer & Kumar, 2012). In Tunisia coastal waters, the chemical composition of both sexes of *Diplodus annularis* species were determined, and it was found that during summer, fat content in males were lower than female during spring but higher for both sexes during Autumn (Khitouni *et al.*, 2014).

### 2.5.3 Seasonal Variation on Moisture in Fish

In Karachi fish harbor, Indian Mackerel (*R. kanagurta*) was investigated and it was discovered that, during summer (rainy season) moisture content were higher than in winter (dry season) (Nisa & Asadullah, 2011). These results agrees with those reported in Oman by Ali *et al.* (2013) that moisture, ash, fatty and fatty acids contents were affected by seasonal variability except for protein and minerals contents. This was supported by Ozogul *et al.* (2011) that, spawning period may influence the content of lipid and moisture in fish.

The observed results of moisture contents in Catfish collected in Jebba Basin, Nigeria was found that, in dry season the content were low (4.24 ± 1.04) and high (5.63 ± 1.27) in wet season (Adelakun *et al.*, 2017). Similar findings were noticed by Paul *et* *al.*(2015). Contrasting results of moisture contents were observed in hake (*Merluccius merluccius*) and Red mullet (*Mullus barbatus*) from Turkey (Tulgar & Berik, 2012). Likewise for Kawa kawa (*Euthynnus affinis*) and frigate tuna (*Auxis thazard*) collected in India by (Rani *et al.,* 2016). Similar observation were found in catfish from Mississippi where a decrease of moisture contents during wet season were obtained by Mustafa & Medeiros (1985). According to the study done by Boran & Karacam (2011) in Turkey, it was found that shad had low moisture content and high fat content than the other species giving out high energy production upon consumption.

### 2.5.4 Seasonal Variation on Ash in Fish

The lower content of ash indicates a lower mineral contents (Dawodu *et al.,* 2012). According to Davies *et al.* (2017), ash content in fish food indicates total minerals contained in it. It was earlier reported by Solanki *et al.* (1976) who worked in silver pomfret (*Pampus argenteus*) of different sizes that were analyzed during winter. In that study, there was no significant differences in ash contents seen during the period of three consecutive months Nov 1969 - Jan 1970. The results of Solanki *et al.* (1976) on the increase of ash content with size was in contrast with the study of Paul *et al.* (2015) where ash content were higher in smaller fish than bigger ones.

In Mesopotamian Catfish, Olgunoglu *et al.* (2014) from Turkey the observed results of ash contents in wet weight (0.93 g/100g) during spring and decreased in winter to (0.67 g/100g). The results of Catfish collected from Upper Jebba Basin in Nigeria showed higher (6.10 ± 0.85 %) concentration of ash in dry season while low concentration of ash was seen in wet season (4.66 ± 0.46 %) (Adelakun *et al.,* 2017). Similar observations were found by Nisa & Asadullah (2011) in Indian mackerel from

Pakistan where ash content was high (1.35 %) in March and low (0.89 %) in December. These results were in conformity with the analysis of catfish from Mississippi (Mustafa & Medeiros, 1985) where ash content in rainy season were lower than in dry season.

In India along Thoothukudi coast where rain falls between July and September, ash contents in sardine (*S. gibbosa*) were lower than during dry season. The results of Tulgar & Berik (2012) from Turkey deviated from other researchers where ash content in Red mullet and Hake were higher in winter during rain than in dry season. Nargis (2006) also found high ash content during rainy season in *Anabas testudineus* from Bangladesh.

### 2.5.5 Seasonal Variation on Carbohydrates in Fish

Carbohydrates are excellent sources of energy and carbon in feed formulations. Primary determinant of carbohydrates' presence are biological factors such as natural feeding habit and evolutionary adaptation. It was reported that, seasonal variation may affect the amount and quality of fish feed also number of movements of the fish (Murray & Burt, 1969). There are fishes that have higher amylase activity, intestinal glucose uptake capacity and control of glycaemia, these are the herbivorous and omnivorous fishes (Kamalam & Panserat, 2016).

Carbohydrates is generally low in fish because there was no remarkable contribution of glycogen in marine animal's reserve (Kamalam & Panserat, 2016). Carbohydrates was determined by using the equation basing on the differences from other components of proximate composition = 100% - (protein % + fat % + ash % + moisture %).

## 2.6 Effects of Processing Methods on Nutrient Composition

Cooking is one of the processing methods where heat is introduced to the fish product and a use of liquid may be a cooking medium. Cooking enhance flavor, digestibility, palatability, color, texture and taste. However it may affects and modify the concentration of some nutrients due to loss or gain of moisture or fats (Gokoglu *et al.*, 2004; Rosa *et al.,* 2007; Stanek *et al.*, 2016). It may improve or impair the nutritional value depending on the method of cooking (Zabadi & Tukura, 2016). It can also cause changes in solubility and nutritional quality of fish (Selmi *et al.,* 2010). It also inactivate pathogenic microorganisms and increase shelf life (Tokur, 2007).

Other report by FAO (2001) stated that during processing of fish, nutrients are preserved in an appreciable quantity. This occurs by interrelating the chemical and physical reactions that may vary depending on time and temperature. It may have a distortion effects on nutrients but may have an important role on fish nutrients (Badiani *et al.,* 2013). It may cause loss of nutrients during cooking (Aberoumand, 2014) or generation of undesirable compounds (Zabadi & Tukura, 2016). The effect of cooking, products are related to the size and composition of the fish (Gall *et al.,* 1983). It was reported that, protein denaturing increase digestibility of food at the same time decreases of labile thermo compounds and polyunsaturated fatty acids (Ktari *et al.*, 2015). It is imperative to conserve nutrients of food products and retention of nutrients in cooked fish which is a major consumer concern to obtain maximum nutritional quality.

### 2.6.1 Effect of frying on mineral content

Frying is one of the cooking methods where fish is deep soaked or shallow submerged in oil using a frying pan at a temperature above boiling point of water, usually 120 – 180 $^{o}$C. This makes a significant change on the food constituents. Frying of fish makes the fish high caloric value to become nutritious and favorably compared to other cooking methods such as baking, grilling and boiling. On frying, the fats replaces the water molecules (moisture) thus makes the food more palatable (Ma *et al.*, 2016). However, the absorption rate of oil differs from one muscle to the other (Musaiger & D'Souza, 2008).

In addition, Lomolino *et al.* (2016) reported that, fish species differences results into variation of mineral concentration when cooked. This was proved by the research of Steiner-asiedu *et al.* (1991) in flat sardine (*Sardinella* sp.), sea bream (*Dentex* sp.), freshwater fish tilapia (*Tilapia* sp) were fried and resulted into variations of mineral content. All mineral (potassium, sodium, magnesium and calcium) contents decreased in flat sardine when fried. In sea bream, all minerals increased in content except potassium while in tilapia, the sodium contents increased and other minerals declined after frying. To some extent, these results were not in agreement with the findings of Hosseini *et al.* (2014) where only potassium and calcium contents were higher while a loss of sodium and magnesium contents was remarked. Ghidurus *et al.* (2010) studied on minerals in fried fish and recorded that, variation of minerals in fried fish was insignificant as the minerals are soluble only in trace amounts in the frying oil.

The former results are in agreement with the study done by Gall *et al.* (1983) on fried mackerel and reported that, no changes of minerals contents. The incident was accepted in the statistical test although there was a decrease of magnesium and calcium mineral contents in fresh *Oreochromis karongae* from Lake Malawi (Kapute & Sainani, 2017). However, some fishes like low fat fish and red snapper may have a decreased content of sodium, potassium and magnesium when fried. According to Koubaa *et al.* (2012) only the contents of potassium increased upon frying the red mullet (*Mullus barbatus*) fillets from Tunisia while sodium, calcium and magnesium decreased.

Table 2.1 Mineral variation in fried and boiled *C. gariepinus*

| **Minerals** | **Raw (mg/Kg)** | **Fried (mg/Kg)** |
|---|---|---|
| **K** | 1817 ±132 | 2373 ± 48.11 |
| **Na** | 308 ± 0.35 | 451 ± 3.86 |
| **Ca** | 40.1 ± 0.08 | 70.9 ± 4.02 |
| **Mg** | 184 ± 18.5 | 248 ± 7.21 |

A research done by Ersoy & Özeren (2009) in African catfish (*C. gariepinus*) from Turkey, had results that are summarized in Table 2.1. The mineral contents of sodium, potassium, calcium and magnesium showed a significant difference at ($p < 0.05$) where there was an increase in contents upon frying due to water loss.

Asian sea bass (*Lates calcarifer*) from Malaysia was studied by Marimuthu *et al.* (2014) and determine the effects of frying on mineral elements. The results (Table 2.2) showed an increase of sodium and potassium while a decrease of calcium and magnesium. These results are in agreement with Ersoy & Özeren, (2009) in the potassium and sodium minerals except in calcium and magnesium contents.

Table 2.2 Mineral variation in fried and boiled *L. calcarifer*

| **Minerals** | **Raw (mg/Kg)** | **Fried (mg/Kg)** | **Boiled (mg/Kg)** |
|---|---|---|---|
| **K** | 3,052± 96.04 | 3,349 ± 245 | 2,858 ± 65.34 |
| **Na** | 232 ± 17.32 | 276 ± 30.88 | 207 ± 22.73 |
| **Ca** | 577 ± 34.59 | 168 ± 23.35 | 545 ± 14.32 |
| **Mg** | 352 ± 35.54 | 274 ± 13.64 | 246 ± 15.15 |

There was an effect of minerals in frying *Oncorhynchus mykiss* (Gokoglu *et al.,* 2004). Results of this study (Table 2.3) concur with the study of Marimuthu *et al.* (2014) but disagree with those of Ersoy & Özeren (2009) on the contents of magnesium and calcium.

Table 2.3 Mineral variation in fried and boiled *O. mykiss*

| **Minerals** | **Raw (mg/Kg)** | **Fried (mg/Kg)** | **Boiled (mg/Kg)** |
|---|---|---|---|
| **K** | 3060 ± 56.8 | 3201 ± 95.2 | 2417 ± 74.2 |
| **Na** | 455 ± 24.5 | 493 ± 1.09 | 335.54 ± 7.81 |
| **Ca** | 632 ± 136 | 147 ± 19.0 | 609 ± 111 |
| **Mg** | 409 ± 13.2 | 268 ± 0.58 | 242 ± 5.99 |

### 2.6.2 Effect of boiling on mineral content

Boiling is another processing method of wet cooking that may lose minerals caused by leaching during boiling or may alter the nutrient components (Ghidurus *et al.*, 2010). Findings from Gokoglu *et al.* (2004) in *O. mykiss* from Turkey showed a decrease of the mineral contents (Table 2.3). These results are in agreement with Marimuthu *et al.* (2014) from Malaysia who worked on Asian sea bass (*Lates calcarifer*). Variation of minerals was also determined in *Kutum roach* by Hosseini *et al.* (2014) that also agreed with the results of Gokoglu *et al.* (2004) done in *O. mykiss* in Table 2.3.

The results of researchers Asghari *et al.* (2013) from Iran studied *O. mykiss* and recorded an increase of calcium and magnesium concentrations upon boiling. These findings differ from the previous study of Gokoglu *et al.* (2004) although the species were the same. From the results of Asghari *et al.* (2013) there was a greater loss of minerals on boiling compared to other cooking methods and consequently was not suggested to be a good method of mineral uptake. In a recent study by Cristelle *et al.* (2018) from Cameroon, the results of four fish species showed a decrease of minerals while calcium increased except in one of the species. The loss of sodium and potassium contents was also observed by Hosseini *et al.* (2014) in *Rutilus frisii kutum* collected from Iran while calcium and magnesium contents were higher than those found in raw fish. An inverse finding of slight decrease of magnesium and no changes in calcium minerals contents were observed in boiled *Oreochromis karongae* from Lake Malawi (Kapute & Sainani, 2017).

It was also known that, mineral content varies depending on the cooking conditions such as the time, temperature and cooking medium (Ersoy *et al.*, 2006). Variation of mineral contents do not occur as a results of heating, but are usually leached if cooked in boiling water as supported by Zabadi & Tukura (2016).

### 2.6.3 Effect of frying on proximate composition

Frying is a commonly practiced process that is fast and cause aromatic flavor making it having a better taste than boiled or steamed food (Nurjanah *et al.*, 2016). Other scientists Saguy & Dana (2003) commended on frying method and reported that, the palatability of fried food is elated to organoleptic and sensory characteristics that include the texture, appearance and flavor. Water contents in fried mackerel were recorded before 76.6 % and after frying 20.39 % (Rahman *et al.*, 2012). It was evident that, exchange of water vapor and moisture by evaporation with oil used for frying replaces fat molecules thus increasing fat content. This was supported by the researchers (Saguy & Dana, 2003; Gokoglu *et al.* 2004; Weber *et al.* 2008) stating that, there was anti-oxidation and not just thermal oxidation.

The effect of frying fish tissues is predominantly in proximate composition especially fat and protein (Nurjanah *et al.*, 2016). Frying reduces the fish platelets aggregation capacity by altering the bioactivity of the fish lipid fraction (Ansorena *et al.*, 2010). This cooking method may lead to increase in fat content in the fried fish where the modification is related to the quicker change of fish temperature. Nevertheless, (Musaiger & D'Souza, 2008) insisted on avoidance of excessing frying that causes excessive absorption of oil (Brannan *et al.*, 2014).

It was also reported by Ayinsa & Maalekuu (2013) that, there was high crude fat content in fried fish, where similar results were observed by Gokoglu *et al.*, (2004). The researchers Mustafa &Medeiros (1985) got the same results of decrease of water content (moisture) while fats, protein and ash increased in content. This report was also supported by Bastías *et al.*, (2017). It was recorded by Weber *et al.* (2008) that, frying compared to baking showed a higher fat content from the raw fish. There was moisture loss and an increase of ash content that indicated total minerals contained (Modibbo *et al.*, 2014). Protein content increased in fried mackerel (*Rastrelliger kanagurta*) due to loss of moisture as supported by Ansorena *et al.* (2010) when frying was done in fillets of cod (*Gadus morhua*) and farmed salmon (*Salmo salar*).

It was reported that, proximate composition contents in raw tissues of *O. mykiss* were as follow: moisture (76 %), protein (20 %), lipid (1.8 %) and ash (0.9 %) (Asghari *et al.*, 2013). On frying, proximate contents occurred as follows; moisture (59.51 %), protein (30.42 %), fat (9.25 %) and ash (1.48 %). These results indicated an increase of protein, lipid and ash contents while moisture content decrease. It was reported that, a loss of moisture causes an increase of protein indicating an inversely proportion to moisture as suggested by Banu *et al.,* (2016).

Bastías *et al.* (2017) recorded that the loss of moisture concentration and an elevated value of protein, fat, and ash was significant in cooked fish. Contradicting results were obtained by Ayinsa & Maalekuu (2013) when frying red fish collected from Ghana. The findings showed a protein and moisture contents decrease, increase of fat contents while no changes in ash content. However, the authors Daramola *et al.* (2007) added that fish crude protein was inversely proportional to fat contents. The decrease of protein content in fried fish was explained by the researchers Ayinsa & Maalekuu (2013) that there was a decrease of protein digestibility during frying therefore reduces the crude protein contents. The incident of increased ash content and loss of water content upon frying corresponds with the increase of other nutrients such as minerals.

Table 2.4 Variation of proximate contents in fried *C. gariepinus*

| Processing methods | Protein (%) | Moisture (%) | Fat (%) | Ash (%) |
|---|---|---|---|---|
| Raw | 16.2 ± 0.66 | 76.8 ± 0.87 | 5.02 ± 0.49 | 0.83 ± 0.10 |
| Fried | 20.0 ± 1.01 | 70.7 ± 0.25 | 8.02 ± 1.68 | 1.15 ± 0.13 |

The results of Marimuthu *et al.* (2014) showed a decrease in moisture content while protein, lipid and ash content increase on fried *L. calcarifer* was in agreement with the studies (Ersoy & Özeren, 2009) in Table 2.4.

### 2.6.4 Effect of boiling on proximate composition

Boiling of fish is one of the preparation process ready for consumption likewise a short-term preservation method whereby heat through boiling water is transmitted to the food. A study of boiled fillets of Silver catfish (*Rhamdia quelen*) was done by Weber *et al.,* (2008). The results showed an increase of protein concentration while moisture contents decreased indicating a reduction of moisture making the protein to be more evident. These results conformed to those reported by Farag (2013); Asghari *et al.,* (2013); Huque *et al.,* (2014). However, Asghari *et al.* (2013) and Farag (2013) reported contrary to Huque *et al.,* (2014). An increase of lipid contents was recorded in the study of Huque *et al.* (2014) and Asghari *et al.* (2013) contrary to the report of Farag (2013).

The studies performed by Marimuthu *et al.* (2014) in Table 2.5 mentioned that, results of lipid contents in boiled fish showed a decrease while ash contents increased. Incompatible findings of protein and moisture were observed where increased and decreased of protein in Marimuthu *et al.* (2014); Cristelle *et al.* (2018) were observed respectively. Similar contradictory results were reported on both studies of the researchers where an increase and decrease contents of moisture respectively. These results disagreed in boiled *P. indicus* done by Aberoumand & Ziaei-Nejad (2015) where ash concentration declined and an increased protein concentration that was contrary to Marimuthu *et al.,* (2014). Low concentration of protein in boiled fish was observed by Bassey *et al.*, (2014). An increase of lipids was also observed in the study done by Karimian-khosroshahi *et al.*, (2016).

Table 2.5 Variation of proximate contents in boiled *L. calcarifer*

| Processing methods | Moisture (%) | Protein (%) | Lipid (%) | Ash (%) |
|---|---|---|---|---|
| Raw | 67.87 ± 0.68 | 18.2 ± 0.05 | 5.13 ± 0.14 | 0.88 ± 0.07 |
| Boiled | 68.15 ± 3.33 | 20.18 ± 0.10 | 4.76 ± 0.49 | 1.15 ± 0.16 |

## 2.7 Analysis of Proximate Composition

Proximate composition in fish includes moisture, crude protein, Nitrogen Free Extract (NFE), crude lipid, ash, and crude fibre (Ayuba & Iorkohol, 2013). These are potential components in food industry for product development, quality control or regulatory purposes. In order to get error free results during the analysis of proximate composition, the samples must be of a homogeneous and representative.

### 2.7.1 Analysis of Proteins

Nitrogen is used as an index of the protein and is termed crude protein because it represents all of the nitrogen that is in the form of Non-Protein Nitrogen (NPN) such as nitrates, ammonia, urea and single amino acids, as well as the nitrogen present as true protein. Proteins are polymers of twenty different types of amino acids includes Lysine, Aspartic acid, Proline, Cysteine, Threonine, Histidine and many other. In fish, the major structural component is proteins that make its overall texture such as tenderness.

The amount of protein in food was calculated by finding the concentration of nitrogen using a standard method by applying a conversion factor 6.25 which is equivalent to 0.16 g nitrogen per gram of protein (Angell *et al.,* 2016). It was therefore important to know the total concentration, type, molecular structure and functional properties of protein (Alberts *et al.,* 2003). On heating, violent vibration and ultimate breakage of hydrogen bonds that holds the amino acid strands and are weak causes protein denaturing that changes the texture of the product (Marimuthu *et al.,* 2012).

### 2.7.2 Analysis of Lipids

Fats are types of lipid and scientifically known as triglycerides with three free acids bound to a molecule of glycerol. Lipids are organic compounds that are mostly soluble in non-polar solvents, but not in water (hydrophobic) with several function such as energy store for cellular use and others (Wang *et al.,* 2015). Cellular lipids molecular species are combinations of minimal number of building blocks.

### 2.7.3 Analysis of Moisture

Moisture component in fish is given in the mass of materials simply water that diffuses in a relatively small quantity, however the relative percentage is dynamic and therefore not constant. Moisture in fresh fish muscles is tightly bound to the protein in the structure and cannot be removed easily unless the condition or state changes and the protein will be less retained. The moisture in frozen fish will be lost as drip when stored at high temperature (Murray & Burt, 1969).

The state of moisture in food occurs as free water where the water is in its physical state, adsorbed water where the water is held tightly in cell walls or protoplasm and is held tightly to proteins and water of hydration where water is bound chemically (Bradley, 2010). The moisture and low volatile materials are removed by heating. It was recorded that removal of water in food reduces the water activity of the product (Driscoll, 2014).

### 2.7.4 Analysis of Ash

Ash are inorganic residue left behind after oxidation of organic matter in food products or can be defined as all the volatile organic constituents in the sample being burnt off leaving behind the non-volatile mineral elements. Dry ashing is usually found in proximate composition and minerals analysis expressed in dry weight basis while wet ashing is fundamentally oxidation for certain minerals and is expressed in wet weight basis (Marshall, 2010).

### 2.7.5 Analysis of Carbohydrates

Carbohydrate is one of the components in proximate composition of fish. Carbohydrates in edible white fish muscles is generally low at a negligible amount with no significant variation in its content (Lilly *et al.*, 2017). Other researchers also stated that non-protein compounds and carbohydrates were found in small amounts and are usually ignored during analysis (Cui & Wootton, 1988). It was reported that, in glycogen in marine animals, do not add significantly in the reserves of their body giving a low levels of carbohydrates (Lilly *et al.*, 2017).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Description of study area

The Coastline of Tanzania has the length of 1,424 km (Mahudi, 2011) from Kenya border in the northeast at latitude 04°39'S to Mozambique border in the south at latitude 10°28'S. It was also recorded that, the coastal continental shelf is generally narrow with the 200 m depth contour being about 4 km offshore, except for the Zanzibar and Mafia Channels where the shelf extends for approximately 60 km. The sampling locations of the study were the Indian Ocean seaports i.e. Tanga, Dar es Salaam, Mtwara and Bagamoyo (Figure 3.1) that experiences wet and dry season annually.

The hottest period extends between November and February while the coldest period occurs between May and August. In October to December, north and east of Tanzania experience two distinct wet periods with short rain periods and long rains from March to May. The southern, western and central parts experience one wet season that run from October through to May (Karmalkar *et al.,* 2003).

The coastal areas of Tanzania are occupied by communities with more or less the same cultures and traditions. Life-earning activities of the coastal communities are mostly agriculture, livestock keeping and fishing. Industrial activities that involve manufactures of Textile, Furniture, Fertilizers, Soap as well as Mining are also practiced (Francis & Bryceson, 2001; Glauber & Jeppesen, 2014). The industries found in the coastal regions of Tanzania include Cement, Beverages and many others.

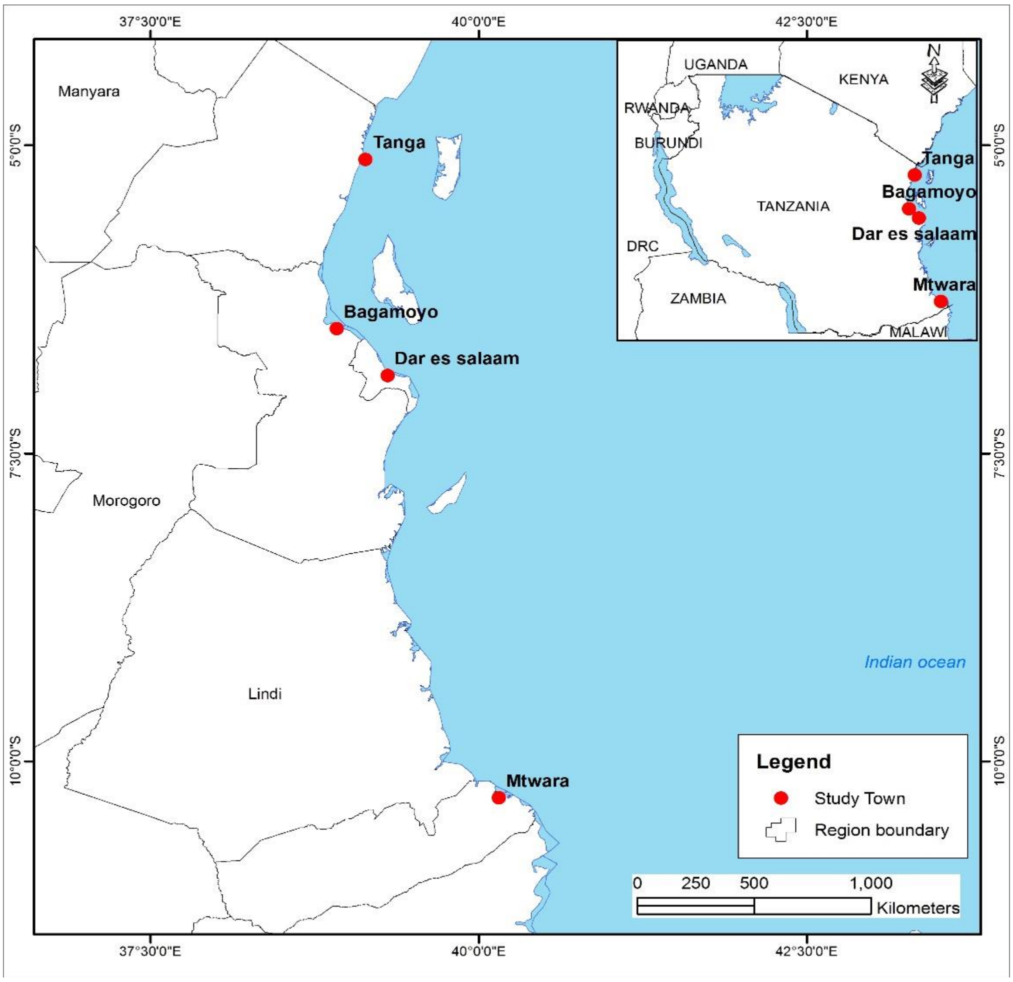


Figure 3.1 Map of the coast of Tanzania showing Study towns

## 3.2 Sampling Techniques

The choice of the fish species for this study was based on their abundance, availability, popularity and ease recognition by consumers. The selected fish species were the African pompano (*Alectis ciliaris*), Snappers (*Lethrinus harak*), Mackerel (*Rastrelliger kanagurta*) and Rabbit fish (*Siganus canaliculatus*). Four fish samples of approximate length (28 - 30 cm) and weight (1.0 - 1.2 kg) for each fish species were purchased from each of the four locations (Tanga, Bagamoyo, Dar es Salaam, and Mtwara) during wet and dry seasons of the year 2017.

Fishes were kept in polythene labeled bags in a cool box filled with ice cubes and transported to the laboratory for processing and analysis. Analysis of mineral contents was done at the Chemistry Department, University of Dar es Salaam (UDSM) while proximate composition analysis was done at the Department of Animal Sciences and Production (DASP), Sokoine University of Agriculture (SUA) in Morogoro.

## 3.3 Sample Preparation

Four fish samples in each of the four species from the four sampling sites were washed several times with tap water to remove slime and adhering blood. The head, scales, gills, tail, fins, bones and internal organs of sampled fish were also removed using clean plastic knife. Only the edible portions of the muscle between the dorsal fin and lateral line were used for analyses. The muscles of the four samples were filleted and randomly mixed together forming a composite. The filleted muscle tissues by species and by location of the catch were then divided into three portions. The first portion were the uncooked therefore considered raw fish fillets while the other two groups were cooked using common household practices, namely frying and boiling.

### 3.3.1 Frying

Deep frying was performed in a domestic frying pan of 2 liters and 25 cm diameter capacity at an initial temperature of 180 °C for 15 minutes (Marimuthu *et al.*, 2014). Sunflower cooking oil extracted from the fatty kernels of *Helianthus annuus* with no other additional ingredients was used as the medium of frying whereby each fish sample was fried using its own cooking oil (oil used only once) then poured out to avoid contamination. After 15 minutes of frying, the fish fillet samples were drained on stainless steel grills, air cooled then packed in labeled aluminum foil in duplicates.

### 3.3.2 Boiling

A saucepan covered with a lid was used in boiling of fish sample in one litre cold water with no additional ingredients at boiling point 99–101°C for 12minutes (Marimuthu *et al.*, 2014). Each sample was boiled using clean (unused) water to avoid contamination and mixture of the nutrients. After boiling, the samples were drained and left to dry and cool then stored in labeled aluminum foil in duplicates.

## 3.4 Sample analysis

The raw and processed (fried, boiled) fish samples were then stored in laboratory refrigerator at -20 °C until analysis. Before analysis, the packed fish samples (raw, fried and boiled) were taken out of the refrigerator and left to thaw. The packages were then oven-dried at 105ºC - 109ºC for 20 hours to a constant weight then each package were ground to pass fine mesh sieve using a mortar and pestle. This is to ensure homogeneity and representative samples are taken for analysis. The fish sample packages were then kept in a desiccator ready for further analyses.

### 3.4.1 Mineral Analysis

Determination of minerals (Potassium, Sodium, Calcium and Magnesium) from the fish samples was done using standard methods (Poitevin, 2016). Analysis was done by first oven-drying the packed and labeled samples at 60 ºC for 24 hours. In each of the dried labeled sample, 1.0 g was digested in conc $HNO_3$/HCl (1:1) mixture followed by addition of 10 mL perchloric acid. The digested sample was then heated using graphite furnace AAS (Perkin-Elmer model 5000 Atomic Absorption Spectrometer) for 10 minutes. Thereafter, the heated digested sample was filtered into 50 mL volumetric flask and diluted to the mark with deionized water prior to analysis using Atomic Absorption Spectrophotometer (AAS).

### 3.4.2 Proximate Composition Analysis

Proximate composition analysis was done using standard methods of Association of Official Analytical Chemists-AOAC (Helrich, 1990).

#### 3.4.2.1 Dry Matter Analysis

Dry matter was analyzed by using clean and dried crucibles that were left to cool in a moisture free desiccator. Each crucible was weighed, labeled ($W_1$) using an analytical balance and recorded. The samples were weighed 2g ($W_2$) in duplicates then put in the labeled crucibles. The labeled crucibles with samples were transferred using tongs to an oven at temperature105 $^{o}$C for 24 hrs until no moisture left. The dried samples in crucibles were removed from the oven and left to cool in a desiccator for 10mins before re-weighed ($W_3$).

The percentage of dry matter was calculated as follows:

$$\frac{\left[\left(W_1+W_3\right)-W_1\right]}{W_2}\times 100=\%DM$$

*Equation 1*

Where; $W_1$ = Weight of empty crucible; $W_2$ = Weight of original sample; $W_3$ = Weight of dried sample and DM = Dry matter

#### 3.4.2.2 Ash Content Determination

Empty crucible was dried in the oven then cooled in the desiccator and weighed ($W_1$). A dried sample of 2g obtained after moisture extraction was put into the crucible and the weight recorded ($W_2$). The crucible with the sample was transferred into a muffle furnace and incinerated at combustion temperature 550 $^{0}C$ for 24 hours. After completion, the residue in crucibles were left to cool in a desiccator and re-weighed ($W_3$). Percentage ash content was computed by carrying out the following calculations:

$$\%Ash\ content = \frac{W_3 - W_2}{W_{2} - W_1} \times 100$$

*Equation 2*

Where; $W_1$ = Weight of empty crucible; $W_2$ = Weight of known amount of sample (fresh) and $W_3$ = Weight of sample residues after incineration

### 3.4.2.3 Crude Protein Determination

The crude protein was analyzed by determining Nitrogen value through the Kjeldahl standard method. Crude protein determination was carried out in three stages which are digestion, distillation and titration.

***Digestion***: The samples were weighed to approximately 0.5 g and placed in labeled digestion tubes that contain 2 digestion tablets and some pumice stones (anti - bumping granules) were also added. A catalyst that consisted a mixer of Potassium Sulphate (10 parts) ($K_2SO_4$), Copper Sulphate (1 part) ($CuSO_4$) and Selenium crystals (0.1 part) (Se) was added estimated ¼ of a teaspoon. It was known that, $K_2SO_4$ raised Sulphuric Acid ($H_2SO_4$) boiling point of 370 $^{o}C$ while $CuSO_4$ and Se crystals speed the process during digestion. Mixing of the sample with catalyst was done by shaking the digestion tube in a circular fashion. Six (6) mls of concentrated Sulphuric Acid ($H_2SO_4$) was added to the solution by using an adjustable dispenser bottle. Digestion tubes fitted at inclined angle in a digestion rack with vapor exhauster were inserted in the Kjeldahl digested system and switched on then heated gently for approximately 1 hour at temperature 420 $^{o}C$ until frothing subsided. In digestion tube, blank solution containing only sulphuric acid and catalyst was also heated. The digested tubes with both sample was left to cool for 30 minutes. The sample solution became colorless then 30 mls of distilled water was added and mixed thoroughly to dilute the Sulphuric acid.

***Distillation***: Distillation process was done by placing the digestion tube containing the sample solution in Kjeltec system then mounting of Sodium Hydroxide (40 %) with Boric acid (4 %) mixed together to a total of 25 mls. The generated ammonia was entrapped as droplets in a receiving solution containing 25 mls of 2 % Boric acid into 5 mls of Bromocresol green/2 drops of methyl red indicator had been placed.

***Titration***: On completion of distillation, titration process was then carried out by the use of standard weak acid HCl (0.01N) added to boric acid with samples while shaking until the colour turned to pink. The amount of nitrogen present in the sample solution was determined by the reading of the weak acid used.

The Nitrogen percentage (% Nitrogen) contained in the samples was calculated by the following formula:

$$\%Nitrogen = \frac{(W_1 - W_2) \times Normality\ of\ HC1 \times N_2 \times 100}{W_3 \times W_4}$$

$$\%Nitrogen = \frac{Titre\ value \times 0.01 \times 14.007 \times 100}{0.5g \times 10\ ml}$$

*Equation 3*

Where: $W_1$ = Volume of HCl titrates sample; $W_2$ = Volume of HCl titrates blank; $W_3$= Sample weight (g); $W_4$ = Volume of known aliquot; $N_2$ = Nitrogen atomic weight and % crude protein = % Nitrogen x 6.25

### 3.4.2.4 Crude Lipid Determination

A standard method stated by Helrich (1990) using the Soxhlet system was used to determine crude lipid in the fish samples. Fish sample of 5 gms ($W_1$) was placed into a pre - weighed labeled extraction thimble ($W_2$). The removal of trace moisture was done by drying the cup in an oven at 105 $^0$C for 30 minutes. Petroleum ether of 40 mls was poured into a labeled extraction cup. The labeled extraction thimble with the sample was fitted tallying with the cups and inserted into extraction unit, the Soxhlet Extraction apparatus at 115$^o$C for 15 minutes. After reflux extraction, the cups with the thimble were left to cool for 45 minutes. The thimble was moved lower and ether was reclaimed using the apparatus by distilling out some ether. Thereafter, the cup with the pure fat contents was cooled in a desiccator and weighed ($W_3$). The percentage crude fat was calculated as follows:

$$\%Crude\ lipid = \frac{W_3 - W_2}{W_1} \times 100$$

*Equation 4*

Where $W_1$ = Weight of sample; $W_2$ = Weight of thimble and $W_3$ = Weight of cup after extraction

### 3.4.3 Data Analysis

Data from this study were analyzed using IBM SPSS package (Version 20) where mean and standard deviations were determined. An independent sample t- test and Levene's test were used to compare the mineral and proximate contents of the fish between the seasons and different processing methods between (raw and fried, raw and boiled, fried and boiled). The effects of processing methods on mineral and proximate contents in fish species were determined by using Analysis of Variance (ANOVA) in each fish species at 5 % significance level.

Data on concentrations of each mineral and proximate composition in the fish tissue samples and dummies of seasons were fitted in simple linear regression model and parameters estimated. Similarly, data on each mineral and proximate concentration against the processing methods were fitted in multiple linear regression model and parameters estimated.

Since both seasons and processing methods were categorically measured, dummy codes were introduced for each independent variable. Season dummy was assigned the value of 1 for wet season and 0 for dry season. Three dummies were created for the three processing methods. Raw processing dummy was created by assigning the value of 1 for raw state and 0 for others. The boiling method dummy was created by assigning the value of 1 for boiling method and 0 for others. Furthermore, the frying method dummy was created by assigning the value of 1 for frying method and 0 for others. In fitting the data into each of the models, the k-1 dummies rule was applied, where the dummy coded 1 was used as a reference category. This procedure resulted into generation of simple linear regression model equation and multiple regression model for minerals and proximate composition.

The Principle Component Analysis (PCA) as a multivariate analysis technique was used to detect similarities as well as differences in the fish samples.

## 3.5 Quality control

Blank solutions were treated the same as samples to check for contamination throughout the analysis. Standards were also used to compare the values in the fish to the known concentrations.

# CHAPTER FOUR

# RESULTS

## 4.1 Variation of Mineral Contents in Fish at Different Seasons

### 4.1.1 Variation of minerals in *A. ciliaris* between seasons

The mean concentration of Potassium, Sodium, Calcium and Magnesium minerals in tissues of *A. ciliaris* between seasons are illustrated in Figure 4.1a. Low mineral concentration was found in *A. ciliaris* during wet compared to dry season except for calcium mineral. There was a significant variation in mean minerals in *A. ciliaris* between seasons ($p < 0.05$) except in calcium and magnesium minerals.

### 4.1.2 Variation of minerals in *L. harak* between seasons

The mean concentration of Potassium, Sodium, Calcium and Magnesium minerals in tissues of *L. harak* between seasons are outlined in Figure 4.1b. High mineral content was found in *L. harak* during dry compared to wet season except for calcium mineral. There was a significant difference in mean minerals in tissues of *L. harak* between seasons ($p < 0.05$) except in calcium mineral.

### 4.1.3 Variation of minerals in *R. kanagurta* between seasons

The mean concentrations of Potassium, Sodium, Calcium and Magnesium minerals in tissues of *R. kanagurta* between seasons are denoted in Figure 4.1c. The mean mineral concentration was found low in *R. kanagurta* during wet season in comparison to dry season except in Calcium minerals. There was a statistical difference in mean minerals in tissues of *R. kanagurta* between seasons ($p < 0.05$) except in calcium mineral.

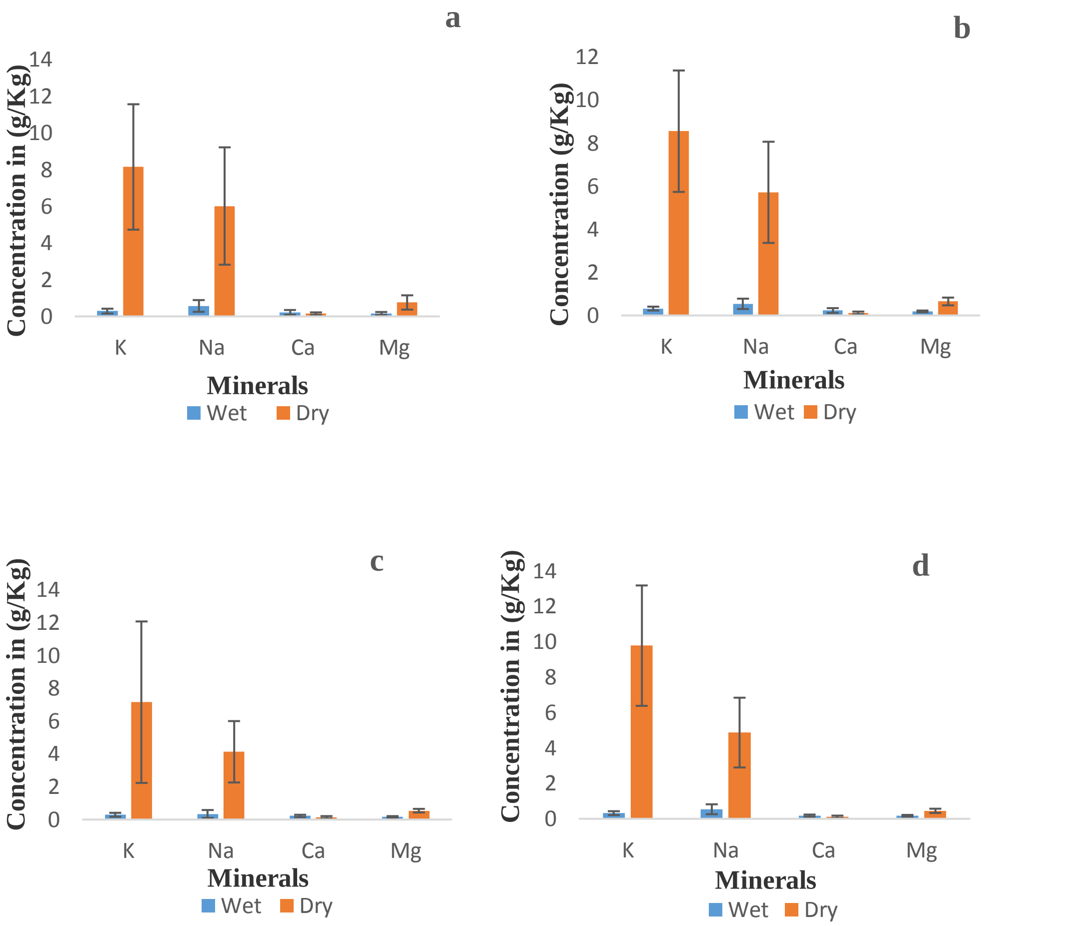


Figure 4.1 Mineral content variation in *A. ciliaris* (a), *L. harak* (b), *R. kanagurta* (c) and *S. canaliculatus* (d) between seasons

### 4.1.4 Variation of minerals in *S. canaliculatus* between seasons

The mean concentration of Potassium, Sodium, Calcium and Magnesium in tissues of *S. canaliculatus* between seasons are shown in Figure 4.1d. Mineral concentration was high in *S. canaliculatus* during dry compared to wet season except in Calcium minerals. There was a statistical significant difference of mean minerals in *S. canaliculatus* between seasons ($p < 0.05$) except in calcium and magnesium minerals.

### 4.1.5 Variation of minerals in different fish species between seasons

The minerals in all analyzed fish species were high during dry compared to wet season except calcium mineral (Table 4.1). There was a statistical significant variation of mineral contents between seasons in all the analyzed species ($p < 0.05$) except calcium in all the fish species and magnesium in *A. ciliaris* and *S. canaliculatus.*

Table 4.1 Variation of mineral contents (g/Kg) in fish species between seasons

| **Mineral** | **Season** | ***A. ciliaris*** | ***L. harak*** | ***R. kanagurta*** | ***S. canaliculatus*** |
|---|---|---|---|---|---|
| **Potassium** | wet | 0.29±0.12 | 0.32±0.09 | 0.30±0.12 | 0.32±0.11 |
| | dry | 8.15±3.49 | 8.56±2.82 | 7.15±4.93 | 9.78±3.40 |
| **Sodium** | wet | 0.57±0.32 | 0.52±0.24 | 0.34±0.24 | 0.54±0.28 |
| | dry | 6.03±3.21 | 5.72±2.35 | 4.13±1.87 | 4.87±1.97 |
| **Calcium** | wet | 0.22±0.14 | 0.23±0.11 | 0.22±0.07 | 0.18±0.06 |
| | dry | 0.15±0.07 | 0.12±0.06 | 0.14±0.07 | 0.11±0.07 |
| **Magnesium** | wet | 0.16±0.08 | 0.19±0.04 | 0.16±0.06 | 0.17±0.05 |
| | dry | 0.76±0.39 | 0.65±0.18 | 0.54±0.11 | 0.45±0.12 |

The overall mean concentration of Potassium, Sodium, Calcium and Magnesium from the selected fish species in wet season were compared to those collected in dry season as shown in Table 4.2.

Table 4.2 Variation of mean mineral contents in fish between seasons

| | **Potassium** | **Sodium** | **Calcium** | **Magnesium** |
|---|---|---|---|---|
| **Wet (g/Kg)** | 0.31±0.11 | 0.51±0.27 | 0.21±0.10 | 0.17±0.05 |
| **Dry (g/Kg)** | 8.41±3.74 | 5.19±2.45 | 0.13±0.07 | 0.60±0.25 |
| **d.f.** | 47 | 48 | 84 | 52 |
| **F - value** | 75.23 | 61.85 | 0.71 | 15.16 |
| **p - value** | 0.00 | 0.00 | 0.40 | 0.00 |

There were low mineral contents in fish species during wet compared to dry season except in Calcium mineral. There was a significant difference in mean mineral contents between the seasons ($p < 0.00$) except in calcium mineral (Table 4.2).

## 4.2 Variation of Proximate Contents in Fish at different Seasons

### 4.2.1 Variation of proximate contents in *A. ciliaris* between seasons

Mean contents of ash and moisture were low in wet season and high in dry season. On the other hand, protein and lipid contents were high in wet season and low in dry season (Figure 4.2a). There was a significant difference in mean proximate contents in *A. ciliaris* between seasons ($p < 0.05$). However, the difference in the mean ash was not significant.

### 4.2.2 Variation of proximate contents in *L. harak* between seasons

The mean contents of crude protein and lipids were high in wet season than in dry season. Whereas, the mean contents of ash and moisture during wet season were lower than those in dry season. (Figure 4.2b). There is a significant difference in the mean lipid and moisture in *L. harak* between seasons ($p < 0.05$) except in ash and crude protein content.

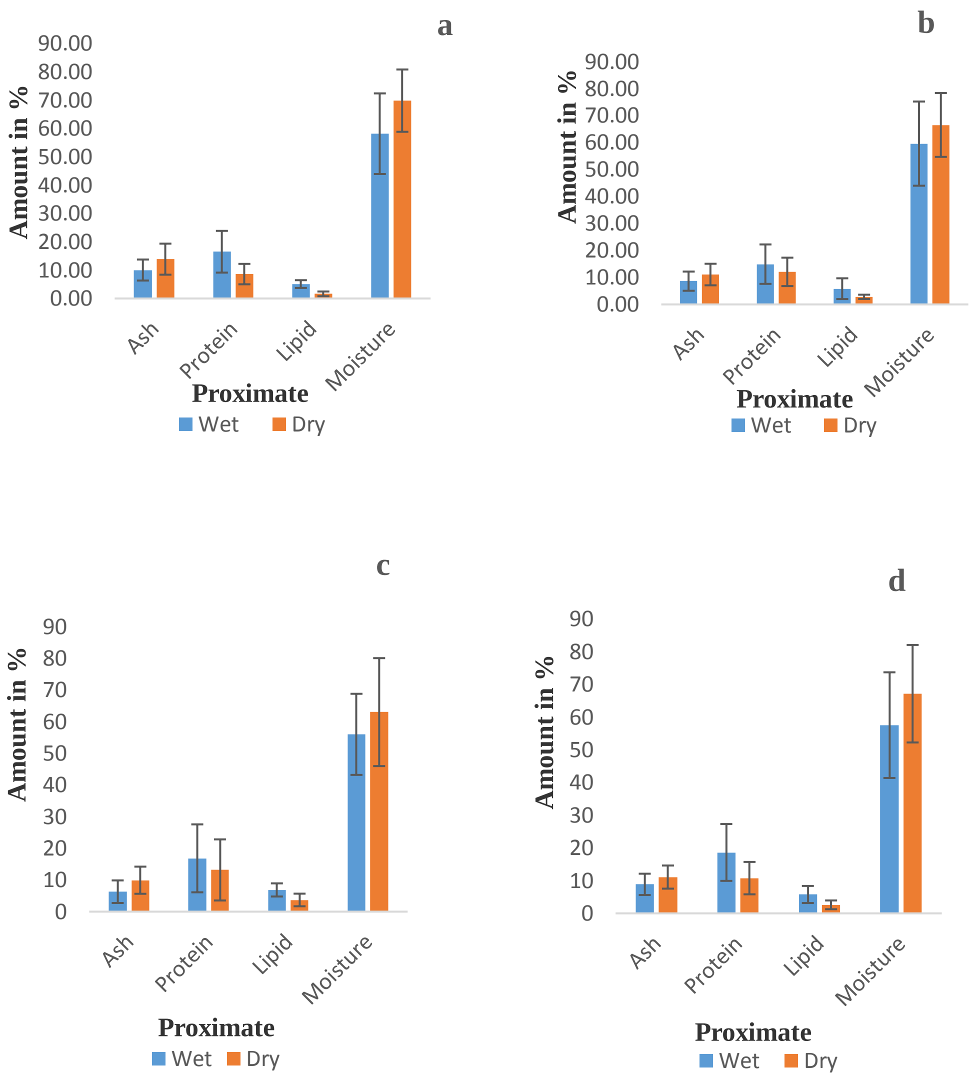


Figure 4.2 Proximate composition variation in *A. ciliaris* (a), *L. harak* (b), *R. kanagurta* (c) *and S. canaliculatus* (d) between seasons

### 4.2.3 Variation of proximate contents in *R. kanagurta* between seasons

The mean contents of ash and moisture during dry season were higher than the contents in wet season. On the other hand, crude protein and lipid were higher in wet season than in dry season (Figure 4.2c). There was a significant difference in mean lipid contents in tissues of *R. kanagurta* between seasons ($p < 0.05$) while no significant difference was observed in other the analyzed proximate contents.

### 4.2.4 Variation of proximate contents in *S. canaliculatus* between seasons

Ash and moisture contents were lower during wet season compared to dry season while crude protein and lipid contents on the other hand were high in wet season compared to during dry season (Figure 4.2d). There was a significant difference in mean proximate contents of *S. canaliculatus* between the seasons ($p < 0.05$) while no significant difference was observed in ash content.

### 4.2.5 Variation of proximate contents in different fish species between seasons

The concentration of proximate composition between seasons in the determined fish species are outlined in Table 4.3. In dry season, all fish species had low contents of both crude protein and lipids, while ash and moisture contents were high (Table 4.3).

Table 4.3 Variation of Proximate contents in fish species between seasons

| **Proximate content** | **Season** | ***A. ciliaris*** | ***L. harak*** | ***R. kanagurta*** | ***S. canaliculatus*** |
|---|---|---|---|---|---|
| Ash (%) | wet | 10.05±3.071 | 8.62±3.56 | 6.30±3.57 | 8.86±3.26 |
| | dry | 13.88±5.48 | 11.08±3.99 | 9.92±4.92 | 11.10±3.54 |
| Crude protein (%) | wet | 16.51±7.37 | 14.93±7.31 | 16.84±10.73 | 18.61±8.69 |
| | dry | 8.62±3.60 | 12.07±5.26 | 13.18±9.65 | 10.77±4.95 |
| Lipid (%) | wet | 5.10±1.37 | 5.84±3.85 | 6.86±2.08 | 5.78±2.61 |
| | dry | 1.64±0.83 | 2.81±0.79 | 3.69±1.99 | 2.61±1.34 |
| Moisture (%) | wet | 58.18±14.23 | 59.62±15.61 | 56.00±12.81 | 57.53±16.15 |
| | dry | 69.85±11.00 | 66.55±11.84 | 63.03±17.03 | 67.17±14.90 |

There was a statistical significant difference in proximate content for all fish species between seasons ($p < 0.05$) except in ash from all fish species, crude protein in *L. harak* and *R. kanagurta* and moisture in *R. kanagurta.*

The overall mean concentration of Ash, Crude protein, Lipid and Moisture in the selected fish species during wet season that were compared to those collected in dry season are denoted in Table 4.4. Ash and Moisture concentration in fish collected in wet season were lower than in dry season. Crude protein and Lipid contents were higher in wet season than in dry season (Table 4.4). There was a significant difference in proximate contents in fish species between seasons ($p < 0.05$). However, the observed difference in ash contents was not significant ($p > 0.05$).

Table 4.4 Variation of proximate contents in fish species between seasons

| | **Ash** | **Protein** | **Lipid** | **Moisture** |
|---|---|---|---|---|
| **Wet (%)** | 8.46±3.51 | 16.72±4.90 | 5.89±2.62 | 57.81±14.41 |
| **Dry (%)** | 11.49±2.20 | 11.16±3.49 | 2.69±1.32 | 66.64±15.38 |
| **d.f.** | 179 | 190 | 189 | 189 |
| **F - value** | 0.00 | 32.18 | 57.26 | 40.20 |
| **p - value** | 0.98 | 0.00 | 0.00 | 0.00 |

## 4.3 Changes of Mineral Contents due to Processing Methods

### 4.3.1 Variation of mineral contents in fish species due to processing methods

Variation of mean mineral levels in selected fish species due to processing methods are presented in Figure 4.3.

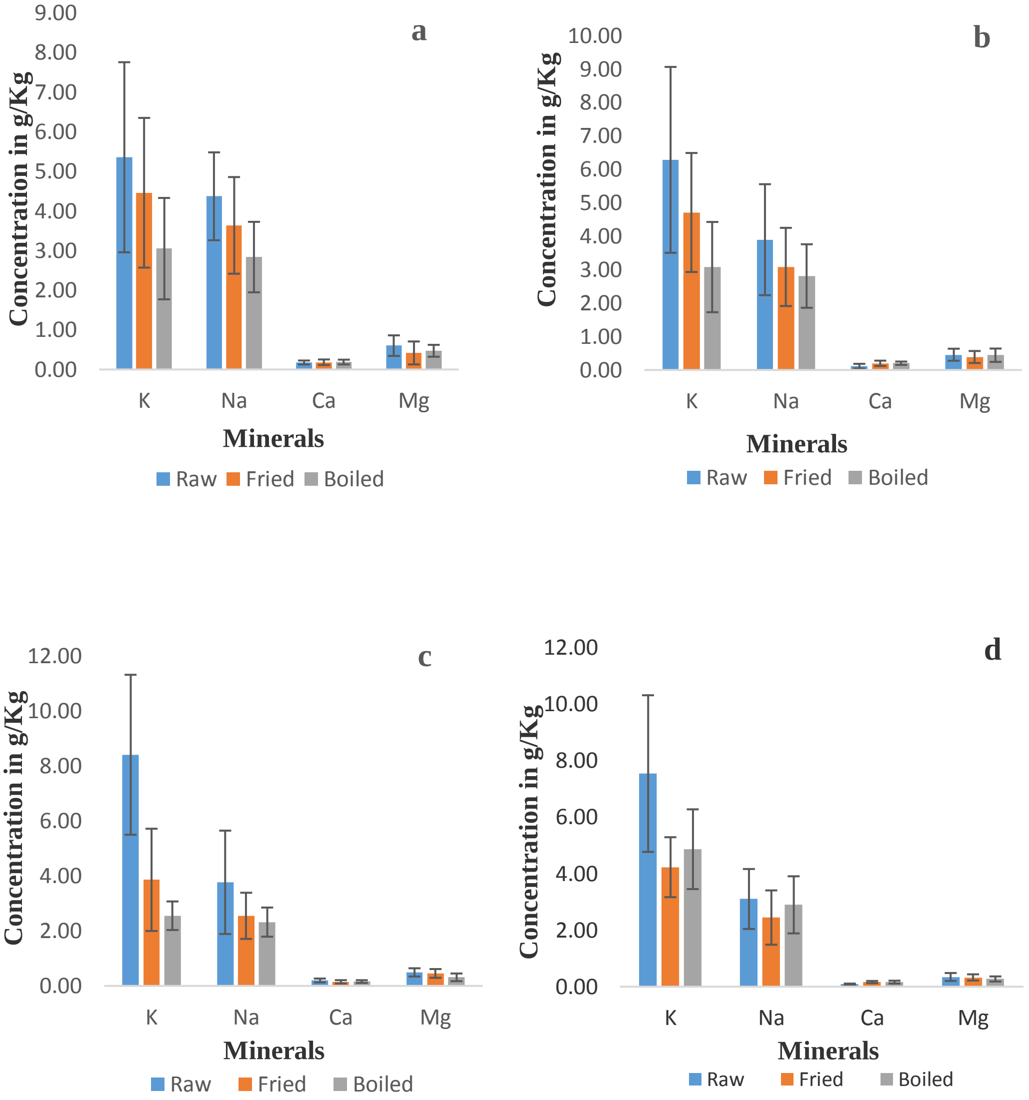


Figure 4.3 Mineral contents variation in *A. ciliaris* (a), *L. harak* (b), *R. kanagurta* (c) and *S. canaliculatus* (d) among processing methods

Potassium and Sodium contents in all selected fish species decreased by varying processing methods with lower values observed using boiling method except in *S. canaliculatus* (Figure 4.3d). Whereas Calcium contents were more or less the same, magnesium contents decreased more when using frying method as shown in *A. ciliaris* and *L. harak* (Figures 4.3a and b) and not in *R. kanagurta* and *S. canaliculatus* (Figures

4.3c and d). The observed variations of the minerals in the selected fish species were not statistically significant ($p > 0.05$).

### 4.3.2 Variation of mineral contents due to processing methods in fish species

The mineral concentration in fish species under processing methods are presented in Table 4.5.

Table 4.5 Changes of minerals in fish species due to processing methods (g/Kg)

| Mineral | Processing method | *A. ciliaris* | *L. harak* | *R. kanagurta* | *S. canaliculatus* |
|---|---|---|---|---|---|
| **potassium** | raw | 5.36±2.40 | 6.29±2.78 | 8.41±2.91 | 7.54±2.77 |
| | frying | 4.46±1.89 | 4.71±1.78 | 3.86±1.86 | 4.23±1.06 |
| | boiling | 3.05±1.28 | 3.08±1.35 | 2.55±0.52 | 4.86±1.41 |
| **sodium** | raw | 4.37±1.11 | 3.90±1.66 | 3.77±1.88 | 3.10±1.06 |
| | frying | 3.64±1.22 | 3.08±1.17 | 2.55±0.84 | 2.44±0.96 |
| | boiling | 3.64±0.89 | 2.81±0.95 | 2.32±0.53 | 2.90±1.01 |
| **calcium** | raw | 0.18±0.05 | 0.11±0.07 | 0.20±0.07 | 0.09±0.02 |
| | frying | 0.18±0.07 | 0.20±0.08 | 0.15±0.06 | 0.16±0.04 |
| | boiling | 0.19±0.06 | 0.20±0.05 | 0.16±0.05 | 0.16±0.05 |
| **magnesium** | raw | 0.60±0.26 | 0.46±0.18 | 0.49±0.15 | 0.34±0.14 |
| | frying | 0.42±0.29 | 0.39±0.18 | 0.45±0.16 | 0.33±0.11 |
| | boiling | 0.47±0.15 | 0.44±0.20 | 0.31±0.14 | 0.27±0.09 |

Generally, mean Potassium Sodium and Magnesium contents in all fish were decreasing by varying processing methods (raw > fried > boiled). An exception was observed in boiled *S. canaliculatus* where Potassium and Sodium contents were higher than in fried *S. canaliculatus*. However, Calcium contents in all fish were more or less the same even after changing the processing method. Lowest Magnesium contents was observed in fried *L. harak* and *S. canaliculatus* and boiled *R. kanagurta* and *S. canaliculatus.* There was no statistical significant difference in the analyzed minerals in fish species between different processing methods ($p > 0.05$).

The overall mean mineral contents in fried fish were compared to raw and the results are summarized in Table 4.6. Whereas the mean contents of Potassium, sodium and magnesium decreased after frying, while Calcium contents increased. There was no significant difference in mean minerals between raw and fried fish ($p > 0.05$).

Table 4.6 Variation of minerals between fried and raw fish

| **Processing method/ mineral** | **Potassium** | **Sodium** | **Calcium** | **Magnesium** |
|---|---|---|---|---|
| **Raw** (g/Kg) | 7.03 ±3.01 | 3.76±1.28 | 0.14±0.08 | 0.47±0.17 |
| **Frying** (g/Kg) | 4.54 ±1.14 | 2.97±1.04 | 0.18±0.08 | 0.39±0.12 |
| **d.f.** | 53 | 53 | 53 | 53 |
| **F-value** | 5.582 | 0.623 | 0.391 | 1.906 |
| **p-value** | 0.22 | 0.43 | 0.53 | 0.17 |

Similarly, potassium, Sodium and Magnesium contents decreased after boiling while Calcium content increased on boiling (Table 4.7).

Table 4.7 Variation of minerals between boiled and raw fish

| **Processing method/ mineral** | **Potassium** | **Sodium** | **Calcium** | **Magnesium** |
|---|---|---|---|---|
| **Raw** (g/Kg) | 7.03 ±4.01 | 3.76±2.28 | 0.14±0.08 | 0.47±0.37 |
| **Boiling** (g/Kg) | 3.37±1.37 | 2.73±1.61 | 0.18±0.08 | 0.38±0.17 |
| **d.f.** | 38 | 55 | 55 | 55 |
| **F-value** | 24.96 | 1.73 | 0.43 | 0.42 |
| **p-value** | 0.00 | 0.19 | 0.52 | 0.52 |

There was a statistical significant difference in Potassium content between raw and boiled fish ($p < 0.05$) while no significant difference was observed for Sodium, Calcium and Magnesium contents ($p > 0.05$).

Table 4.8 Variation of minerals between fried and boiled fish

| Processing method/ mineral | Potassium | Sodium | Calcium | Magnesium |
|---|---|---|---|---|
| **Frying** (g/Kg) | 4.54 ±1.14 | 2.97±1.04 | 0.18±0.08 | 0.39±0.12 |
| **Boiling** (g/Kg) | 3.37±1.37 | 2.73±1.61 | 0.18±0.08 | 0.38±0.17 |
| **d.f.** | 84 | 85 | 85 | 85 |
| **F-value** | 4.29 | 0.92 | 1.39 | 0.79 |
| **p-value** | 0.61 | 0.95 | 0.99 | 0.98 |

Whereas Potassium, Sodium and magnesium contents were higher in fried fish than in boiled fish, Calcium contents were similar. There was no significant difference of mineral content between fried and boiled fish ($p > 0.05$).

## 4.4 Changes of Proximate Contents due to Processing Methods

### 4.4.1 Variation of proximate contents in fish species due to processing methods

The mean proximate contents in tissues of raw fish were compared with cooked and presented in Figures 4.4. The mean ash contents in all fish species slightly decreased in boiled fish but were more or less similar in raw and fried fish. Crude protein and lipid contents in all fish species increased in both processing methods, more so in fried fish. On the other hand, moisture contents in all fish species decreased in both processed fish, more so in fried fish (Figure 4.4). With an exception of ash content in

A. *ciliaris* and *L. harak*, there was significant difference of proximate contents between raw and cooking methods ($p < 0.05$).

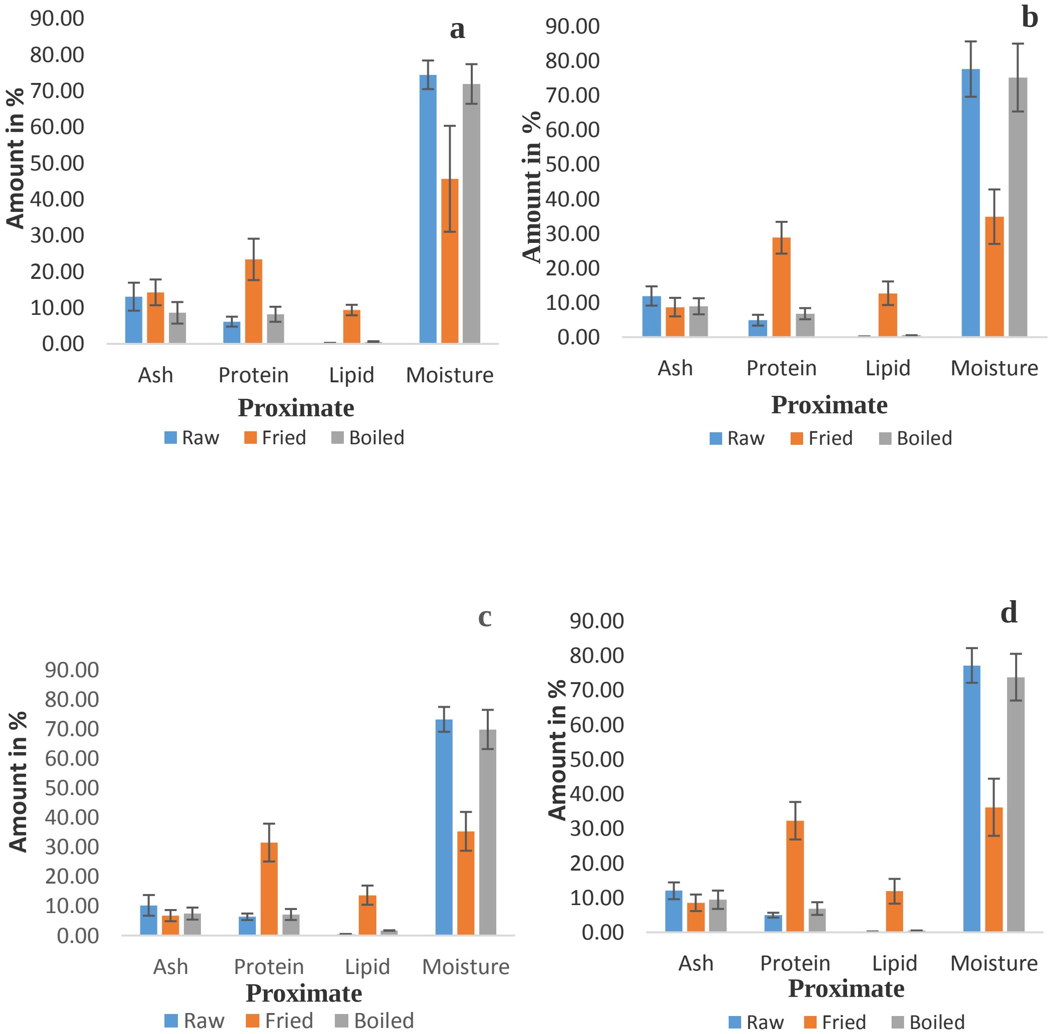


Figure 4.4 Proximate contents variation in *A. ciliaris* (a), *L. harak* (b), *R. kanagurta* (c) and *S. canaliculatus* (d) among processing methods

### 4.4.2 Variation of proximate contents due to processing methods in fish species

The overall proximate contents in the analyzed fish species under processing methods are presented in Table 4.9.

Table 4.9 Changes of proximate contents in fish species due to processing methods

| Proximate content | Processing method | *A. ciliaris* | *L. harak* | *R. kanagurta* | *S. canaliculatus* |
|---|---|---|---|---|---|
| Ash (%) | raw | 13.05±3.87 | 11.92±2.79 | 10.23±3.51 | 12.00±2.43 |
| | frying | 14.25±3.57 | 8.70±2.69 | 6.75±1.89 | 8.53±2.39 |
| | boiling | 8.59±2.98 | 8.94±2.33 | 7.44±2.04 | 9.41±2.65 |
| Crude protein (%) | raw | 6.15±1.37 | 4.91±1.56 | 6.36±1.11 | 4.96±0.70 |
| | frying | 23.37±5.73 | 28.79±4.60 | 31.51±6.41 | 32.25±5.42 |
| | boiling | 8.19±2.08 | 6.80±1.62 | 7.14±1.86 | 6.87±1.83 |
| Lipids (%) | raw | 0.20±0.08 | 0.16±0.16 | 0.48±0.05 | 0.22±0.04 |
| | frying | 9.36±1.45 | 12.71±3.42 | 13.69±3.25 | 11.87±3.58 |
| | boiling | 0.55±0.19 | 0.53±0.03 | 1.70±0.09 | 0.49±0.05 |
| Moisture (%) | raw | 74.45±3.96 | 77.64±8.02 | 73.26±4.22 | 77.13±5.01 |
| | frying | 45.68±14.66 | 34.897.89 | 35.32±6.58 | 36.14±8.25 |
| | boiling | 71.91±5.48 | 75.18±9.83 | 69.84±6.84 | 73.73±6.75 |

Whereas crude protein and lipid contents were high in fried fish while moisture content was low in all fish samples. Raw fish had high ash content in all the fish except *A. ciliaris* where fried fish had higher ash contents. There was a significant difference of proximate contents in cooked fish when compared to raw ($\rho < 0.05$) except ash in *A. ciliaris* and *L. harak*. The overall mean proximate contents in fried fish samples were compared to raw fish and summarized in Table 4.10. Ash and moisture contents in fried fish and were lower compared to the contents in raw fish. The fried fish showed high protein and lipid contents compared to raw fish. There was a significant difference in proximate contents between fried and raw fish ($p < 0.00$) except in ash.

Table 4.10 Variation of proximate contents between fried and raw fish

| Processing method/ proximate | Ash | Protein | Lipid | Moisture |
|---|---|---|---|---|
| **Raw** (%) | 11.80±4.51 | 2.84 ±1.09 | 0.27±0.21 | 75.62±3.65 |
| **Frying** (%) | 9.57±7.99 | 5.21±2.73 | 11.89±7.66 | 38.06±19.28 |
| **d.f.** | 125 | 81 | 62 | 66 |
| **F-value** | 1.13 | 64.91 | 158.59 | 108.67 |
| **p-value** | 0.29 | 0.00 | 0.00 | 0.00 |

The changes of proximate contents in boiled fish compared to raw fish are outlined in Table 4.11. Ash, protein and moisture contents in boiled fish were lower compared to the contents in raw fish. The boiling increased the lipid content compared to raw fish. Whereas there was a significant difference in lipid content between raw and boiled fish ($p < 0.05$) and no significant difference in other proximate contents between raw and boiled fish (Table 4.11).

Table 4.11 Variation of proximate contents between boiled and raw fish (%)

| **Processing method/ proximate** | **Ash** | **Protein** | **Lipid** | **Moisture** |
|---|---|---|---|---|
| **Raw (%)** | 11.80±4.51 | 2.84 ±1.09 | 0.27±0.21 | 75.62±3.65 |
| **Boiling (%)** | 8.61±4.76 | 2.21±0.99 | 0.80±0.14 | 72.71±5.34 |
| **d.f.** | 125 | 125 | 70 | 109 |
| **F-value** | 0.39 | 1.36 | 13.16 | 6.81 |
| **p-value** | 0.53 | 0.25 | 0.00 | 0.10 |

The differences of proximate contents between fried and boiled fish samples are presented in Table 4.12.

Table 4.12 Variation of Proximate contents between boiled and fried fish

| **Processing method/ proximate** | **Ash** | **Protein** | **Lipid** | **Moisture** |
|---|---|---|---|---|
| Frying (g/Kg) | 9.57±7.99 | 5.21±2.73 | 11.89±7.66 | 38.06±19.28 |
| Boiling (g/Kg) | 8.61±4.76 | 2.21±0.99 | 0.80±0.14 | 72.71±5.34 |
| d.f | 124 | 125 | 124 | 124 |
| F-value | 0.67 | 133.21 | 127.35 | 189 |
| p-value | 0.42 | 0.00 | 0.00 | 0.00 |

Ash, protein and lipid contents in boiled fish were lower compared to the contents in fried fish and moisture in boiled fish was higher than in fried fish. Whereas there was a significant difference in proximate contents between fried and boiled fish ($p < 0.05$) and insignificant difference in ash content between fried and boiled fish (Table 4.12).

## 4.5 Predicting Mineral and Proximate Contents due to Seasons

### 4.5.1 Predicting values of mineral contents due to seasons

Regression analysis was applied to assess the relationship between mineral contents found in tissues of the sampled fish species and seasonal changes. The findings of the study showed that the predictive model values of the minerals in seasons can be determined by the regression equation:

$$Y_i = b_0 + bx + \varepsilon_i$$

*Equation 1*

The predicted model results are presented and summarized in Table 4.13. The variances of the mineral composition in tissues of the fish species between seasons are (67.6 %, for potassium 62 % for sodium 19.3 % for calcium and 55.1 % for magnesium) as explained by the predictive model.

Table 4.13 Linear regression prediction analysis of minerals in fish species between seasons.

| **Mineral** | **$R^2$** | **($b_0$)** | **Wet Dummy variable (*bx*)** | **F value** | **p value** | **Predicted value** |
|---|---|---|---|---|---|---|
| **Potassium** | 0.676 | 8.91 | -8.04 | 172.85 | 0.00 | 0.87 |
| **Sodium** | 0.620 | 5.45 | -4.65 | 136.87 | 0.00 | 0.80 |
| **Calcium** | 0.193 | 0.13 | 0.08 | 20.13 | 0.00 | 0.21 |
| **Magnesium** | 0.551 | 0.62 | -0.43 | 103.20 | 0.00 | 0.19 |

Potassium values were predicted to decrease by 8.04 g/Kg in wet season compared to dry season and the change is expected to be statistically significantly lower ($p < 0.05$). Sodium values were predicted to decrease by 4.65 g/Kg in wet season compared to dry season. The difference is expected to be significantly lower ($p < 0.05$). Calcium contents in wet season were predicted to increase by 0.08 g/Kg compared to dry season and the change is expected to be significantly higher ($p < 0.05$). The quantities of magnesium were predicted to decreases by 0.43 g/Kg in wet season relative to dry season, the change is expected to be significantly lower ($p < 0.05$).

The predictive model values of the minerals found in tissues of the fish species between seasons will vary by 0.87 g/Kg for potassium, 0.80 g/Kg for sodium, 0.21 g/Kg for calcium and 0.19 g/Kg for magnesium. There is a statistical significant contribution of seasons ($p < 0.05$) in the variation of mineral contents in the selected fish. This indicates that the model can accurately predict the variation in minerals contents using seasons as a predictor.

### 4.5.2 Predicting values of proximate contents due to seasons

The findings of the study showed that the predictive model value of the proximate composition in seasons can be determined by the regression equation:

$$Y_i = b_0 + bx + \varepsilon_i$$

*Equation 2*

The predicted model results are outlined and summarized in Table 4.14. The unique variances of the proximate composition in tissues of the fish species between seasons are (6.3 % for ash, 4.1 % for crude protein, 5.3 % for lipids and 4.6 % for moisture) as expressed by the predictive model.

Table 4.14 Linear regression prediction analysis of proximate composition between seasons

| **Proximate composition** | **$R^2$** | **($b_0$)** | **Wet Dummy variable ($bx$)** | **F value** | **p value** | **Predicted value** |
|---|---|---|---|---|---|---|
| Ash | 0.063 | 11.50 | -3.04 | 12.69 | 0.00 | 8.46 |
| Crude Protein | 0.041 | 11.16 | 5.56 | 8.18 | 0.00 | 16.42 |
| Lipid | 0.053 | 2.68 | 3.21 | 10.61 | 0.00 | 4.89 |
| Moisture | 0.046 | 66.67 | -8.83 | 9.12 | 0.00 | 57.84 |

In wet season relative to dry season, ash content was predicted to decrease by 3.04 %, and the decrease is expected to be significantly lower ($p < 0.05$). Crude protein values were predicted to increase by 5.56 % in wet season compared to dry season. The increase is expected to be significantly higher ($p < 0.05$). Similarly, Lipid content in wet season was predicted to increase by 3.21 % compared to dry season, the change is expected to be significantly higher ($p < 0.05$). Furthermore, moisture contents in wet season was predicted to decrease by 8.83 % compared to dry season and the change is expected to be statistically significantly lower ($p < 0.05$).

The predictive model values of proximate composition in the fish species between seasons will vary by 8.46 % for ash, 16.42 % for crude protein, 4.89 % for lipid and 57.84 % for moisture. There is a statistical significant contribution of seasons ($p < 0.05$) in variation of proximate contents. This is an indication that, the model can accurately predict proximate contents using seasons as a predictor.

## 4.6 Predicting Mineral and Proximate Contents due to Processing Methods

### 4.6.1 Predicting values of mineral contents due to processing methods

The findings of the study showed that the predictive model values of the minerals due to varying processing methods can be determined by the multiple regression equation:

$$Y_i = b_0 + b_1X_1 + b_2X_2 + \varepsilon_i$$

*Equation 3*

The predicted model results are summarized in Table 4.15. The individual variances of the mineral composition in processed fish species are 9.5 % for potassium, 2.2% for sodium, 3.3 % for calcium and 1.9 % for magnesium as explained by the multiple regression equation.

The model predicted a decrease in potassium values by 2.49 % in fried fish and 3.67 % in boiled fish. The changes predicted was significantly lower ($p < 0.05$). Sodium values were predicted to decrease by 0.79 % in fried fish and 1.03 % in boiled fish. The changes predicted were statistically insignificantly lower ($p > 0.05$). Similarly, the Calcium values were predicted to increase by 0.04 % in both fried and boiled fish. However, the change was expected to be insignificantly higher ($p > 0.05$). The model also predicted a decrease in magnesium values by 0.08 % fried fish and 0.09 % in boiled fish. The predicted changes are expected to be not significant ($p > 0.05$).

Table 4.15 Multiple regression prediction analysis of mineral concentration due to processing methods

| **Mineral** | **Processing Method** | **Model (*b*)** | **t value** | **p value** | **F value** | **$R^2$** | **Constant ($b_0$)** |
|---|---|---|---|---|---|---|---|
| Potassium | Frying Dummy ($b_1$) | -2.49 | -1.95 | 0.05 | 4.29 | 0.095 | 7.03 |
| | Boiling Dummy ($b_2$) | -3.67 | -2.89 | 0.00 | | | |
| Sodium | Frying Dummy ($b_1$) | -0.79 | -0.99 | 0.32 | 0.92 | 0.022 | 3.76 |
| | Boiling Dummy ($b_2$) | -1.04 | -1.31 | 0.19 | | | |
| Calcium | Frying Dummy ($b_1$) | 0.04 | 1.40 | 0.16 | 1.39 | 0.033 | 0.14 |
| | Boiling Dummy ($b_2$) | 0.04 | 1.52 | 0.13 | | | |
| Magnesium | Frying Dummy ($b_1$) | -0.08 | -0.99 | 0.33 | 0.79 | 0.019 | 0.47 |
| | Boiling Dummy ($b_2$) | -0.09 | -1.19 | 0.24 | | | |

Using multiple regression, the predicted mineral values in processing methods is 0.87 g/Kg for potassium, 1.94 g/Kg for sodium, 0.22 g/Kg for calcium and 0.30 g/Kg for magnesium (Table 4.15). There is a statistical significance contribution of processing methods to variation of potassium ($p < 0.05$), and insignificant variation of sodium, calcium and magnesium. The model can accurately predict the variation of Potassium content but not for Sodium, Calcium and Magnesium contents using processing methods as a predictor.

### 4.6.2 Predicting values of proximate contents due to processing methods

The findings of the study showed that the predictive model values of the proximate composition due to varying processing methods can be determined by the multiple regression equation:

$$Y_i = b_0 + b_1X_1 + b_2X_2 + \varepsilon_i$$

*Equation 4*

The predicted model results are summarized in Table 4.16. The individual variances of the proximate composition in the fish species were 4.60 % for ash, 60.6 % for protein, 59.1 % for lipid and 68.3 % for moisture as described by the multiple regression model.

The model predicted a decrease in ash contents by 2.14 % in fried fish and 3.09 % in boiled fish compared to raw fish. The changes are expected to be statistically significantly lower ($p < 0.05$). Crude protein contents were predicted to increase by 23.36 % in fried fish and 1.62 % in boiled fish compared to raw fish. Lipid contents were predicted to increase by 11.62% in fried fish and 0.53% in boiled fish. The predicted moisture contents were predicted to decrease by 37.5% in fried fish and 2.85% in boiled fish. Like crude protein and lipids contents, the change in moisture content is predicted to be statistically significantly for fried fish ($p < 0.05$) and not for boiled fish.

Table 4.16 Multiple regression prediction analysis of proximate contents due to processing methods

| **Proximate content** | **Processing Method** | **Model value (*b*)** | **t value** | ***p* value** | **F value** | **$R^2$** | **Constant ($b_0$)** |
|---|---|---|---|---|---|---|---|
| Ash | Frying Dummy ($b_1$) | -2.14 | -2.03 | 0.04 | 4.55 | 0.046 | 11.71 |
| | Boiling Dummy ($b_2$) | -3.09 | -2.94 | 0.00 | | | |
| Crude protein | Frying Dummy ($b_1$) | 23.36 | 15.31 | 0.00 | 145.15 | 0.606 | 5.63 |
| | Boiling Dummy ($b_2$) | 1.62 | 1.06 | 0.29 | | | |
| Lipid | Frying Dummy ($b_1$) | 11.62 | 14.66 | 0.00 | 135.66 | 0.591 | 0.27 |
| | Boiling Dummy ($b_2$) | 0.53 | 0.67 | 0.50 | | | |
| Moisture | Frying Dummy ($b_1$) | -37.51 | -18.15 | 0.00 | 202.46 | 0.683 | 75.56 |
| | Boiling Dummy ($b_2$) | -2.85 | -1.38 | 0.17 | | | |

Using the multiple regression, the predicted proximate values due to processing methods were 6.48 % for ash, 30.61 % for protein, 12.42 % for lipid and 35.20 % for moisture (Table 4.16). There is a statistical significance contribution of both frying and boiling methods in ash content and frying method to protein, lipid and moisture ($p < 0.05$). The model can accurately predict the variation of ash content only using both processing methods as predictors. The model can only predict accurately the variation of crude protein, lipid and moisture when frying processing method was used as a predictor.

### 4.7 Principal Component Analysis (PCA)

In determining the relationship between minerals, proximate composition, seasons and processing methods, PCA after varimax rotation was employed. A principal component (PC) was considered significant when its eigenvalue was greater than 1. The measured values were used as variables (total 12) with the concentrations of the nutrients in the different sampling stations as objects (total 192). Based on the loading distribution of the variables, the PCA results indicated that the variables can be represented by five principal components (PCs) that accounted for 68.1 % of the total variance in the original data sets (Table 4.17).

Based on the Table 4.17, potassium, sodium and magnesium that contributed 32.8 % of total variances constituted one related group (PC 1), indicating their relationship in origin of effect (season). This is evidenced by the Pearson correlation coefficients (Table 4.18) and the loading plot (Figure 4.5). The Table 4.17 indicated that potassium, sodium and magnesium were significantly correlated ($p = 0.00$), indicative of similar source. Therefore, these minerals were correlated with season.

Table 4.17 Rotated loadings of the Principal components

| **Variable** | **Principal Component (68.1%)** | | | | |
|---|---|---|---|---|---|
| | **PC 1 (32.8%)** | **PC 2 (18.6%)** | **PC 3 (9.1%)** | **PC 4 (4.4%)** | **PC 5 (3.2%)** |
| **Sodium** | **0.914** | 0.011 | 0.045 | -0.134 | 0.009 |
| **Potassium** | **0.910** | 0.029 | 0.102 | 0.039 | 0.029 |
| **Magnesium** | **0.892** | 0.034 | -0.094 | 0.040 | -0.049 |
| **Season** | **0.567** | -0.219 | 0.289 | 0.021 | 0.012 |
| **Moisture** | 0.076 | **-0.955** | 0.163 | -0.015 | 0.028 |
| **Lipid** | -0.097 | **0.944** | -0.103 | 0.015 | -0.048 |
| **Protein** | 0.258 | **0.751** | 0.448 | -0.105 | 0.121 |
| **Ash** | 0.359 | -0.060 | **0.688** | -0.143 | 0.201 |
| **Process** | 0.012 | 0.023 | **0.624** | 0.097 | -0.146 |
| **Calcium** | 0.488 | 0.132 | **-0.551** | 0.015 | -0.047 |
| **Location** | -0.006 | -0.021 | 0.019 | **0.984** | 0.018 |
| **Species** | -0.024 | -0.004 | -0.026 | 0.020 | **0.973** |

Figure 4.5 has also indicated that potassium, sodium and magnesium are related to each other. This clearly indicates that these minerals are significantly affected by season. Table 4.17 also indicates that moisture, lipid and crude protein which contributed 18.6 % of total variance constituted another related group (PC 2), indicating their relationship. This has been shown in Table 4.18 where these variables are significantly correlated to each other ($p = 0.00$).

Ash, processing methods and calcium constituted a third related group (PC 3) (Table 4.17). Figure 4.5 clearly indicated that ash and processing methods are closely related but negatively related to calcium. This is an indication that calcium is inversely related to ash and processing methods.

The fourth and fifth PCs were constituted by location and species, respectively, indicative of the lack of relationship between location and species and the analyzed components (Table 4.17 and Figure 4.5). This imply that the variation of the constituents in fish were independent to location and type of fish species.

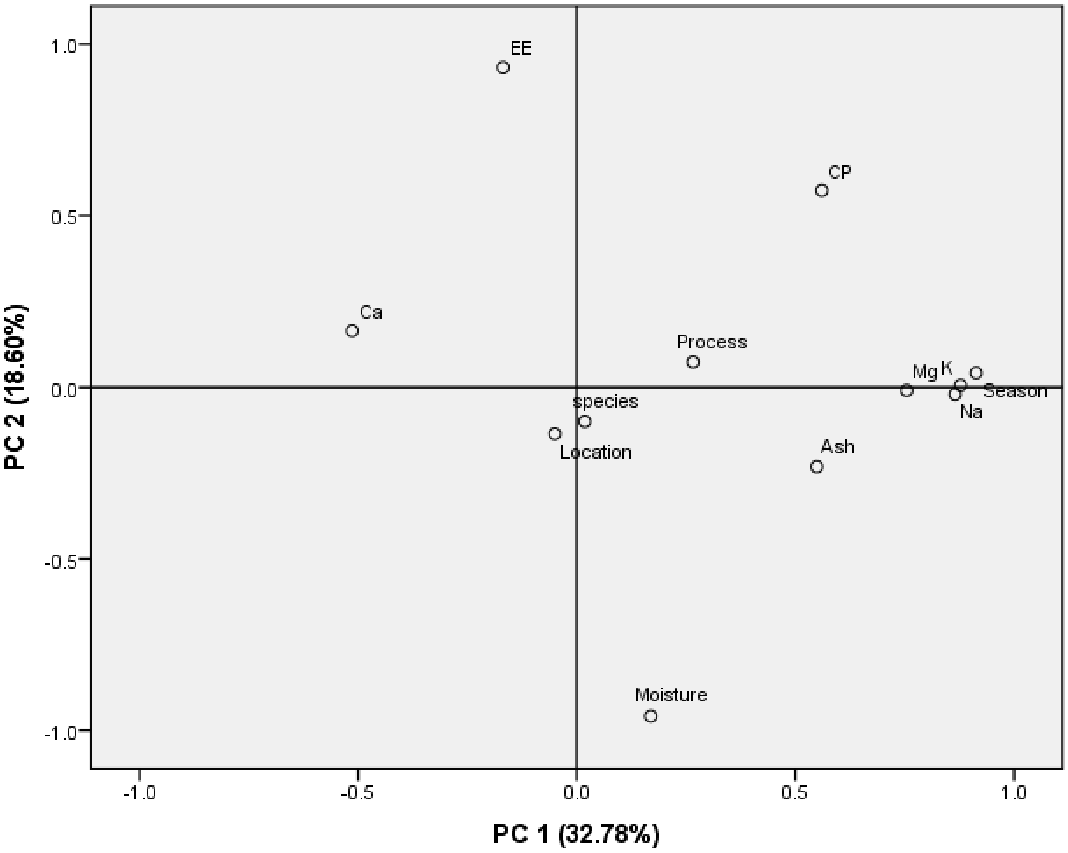


Figure 4.5 A two dimensional score plot for the variables in the study

Table 4.18 Pearson Correlation Coefficients of the analyzed variable

| | Fish species | Ash | Protein | Lipid | Moisture | Potassium | Sodium | Calcium | Magnesium | Processing method | Location | Season |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fish species | 1 | | | | | | | | | | | |
| Ash | 0.077 | 1 | | | | | | | | | | |
| Protein | 0.038 | **0.463** | 1 | | | | | | | | | |
| Lipid | -0.036 | **-0.200** | **0.540** | 1 | | | | | | | | |
| Moisture | 0.011 | **0.286** | **-0.571** | **-0.926** | 1 | | | | | | | |
| Potassium | 0.028 | **0.292** | **0.271** | -0.078 | 0.045 | 1 | | | | | | |
| Sodium | -0.007 | **0.324** | **0.255** | -0.079 | 0.067 | **0.852** | 1 | | | | | |
| Calcium | -0.037 | -0.022 | 0.016 | 0.067 | -0.106 | **0.310** | **0.331** | 1 | | | | |
| Magnesium | -0.045 | **0.242** | ***0.173*** | -0.025 | 0.022 | **0.743** | **0.752** | **0.444** | 1 | | | |
| Processing method | 0.000 | **0.218** | 0.119 | -0.032 | 0.058 | 0.129 | 0.034 | -0.115 | 0.011 | 1 | | |
| Location | -0.003 | -0.061 | -0.073 | -0.004 | 0.012 | 0.006 | -0.129 | -0.003 | 0.012 | 0.000 | 1 | |
| Season | -0.007 | **0.251** | 0.097 | **-0.230** | **0.215** | **0.502** | **0.475** | -0.093 | **0.411** | 0.000 | -.002 | 1 |

Bold means significant correlation at the $\alpha = 0.5$ (2-tailed).

# CHAPTER FIVE

# DISCUSSION

## 5.1 Effects of Seasonal Changes on Mineral Contents in Fish Species

### 5.1.1 Mineral contents in *A. ciliaris*

The concentration of the minerals (potassium, sodium and magnesium) showed a higher concentration predominantly during dry season than in wet (rainy) season except in calcium (Figure 4.1a). The t-test showed a significant difference of minerals on changing seasons ($p < 0.05$) except in calcium and magnesium. The value concentration of calcium in seawater is 400 mg/L and that of magnesium is 1,350 mg/L forming a hardness concentration of over 6,000 mg/L (Boyd, 2015). Nevertheless, the hardness concentration vary depending on environment and time that eventually cause differences in minerals.

Seawater was reported to be the major source of minerals (Noriki, 1978, Bardi, 2010). These minerals are made easily available in solution through the fishes' diet which invariably become assimilated into the body system. In addition, the adult species *A. ciliaris* lives in sandy or near the bottoms and stony formation grounds that may probably not experience the influence of flowing rainwater to dilute their environment. It was noted that, the movement of ocean water aftermath in the proportion of mineral salts remain fairly uniform in all oceans (Fontaine & Wilcock, 2006). Evaporation of seawater leaving salt behind causes variations of mineral salts between locations and parts of the ocean (Bunnet, 1988) depending on the climate.

From this study, the results were similar with the findings of Olgunoglu *et al.,* (2014). These authors were working on Mesopotamian Catfish (*Silurus triostegus*) collected from the fourth largest clay-cored rock in the world Atatürk Dam Lake. The similarities may probably be a result of dilution of the bottom combined soil minerals and clay minerals that occurs during rainfall. In dry season, there would be higher concentration of minerals in the dam due to poor dilution from rain water.

The findings of Khitouni *et al.* (2014) in *Diplodus Annularis* differed from the results of the current study on mineral concentration during wet season. This deviation of results from the present study could possibly be a repercussion of the characteristics of the Gulf of Gabès where the fish were collected. As reported by Enríquez *et al.* (2017), sea-level and erosion were the major problems of Gulf of Gabès. Other related problems were desertification, drought and salinization with addition of inundation and coastal erosion. On top of this, Mediterranean Sea provides a negative water balance that has less inflow (river run-off and precipitation) than evaporation (Talley, 2001).

### 5.1.2 Mineral contents in *L. harak*

The results from this study showed that, the concentration of minerals in dry season was high compared to wet season except in calcium (Figure 4.1b). The statistical t-test showed a significance difference of mineral contents between wet and dry season ($p < 0.05$) and not significant in calcium content. The differences of mineral concentration between the seasons may probably be induced by some parameters such as alkalinity and pH that would possibly affect the water hardness where the fish inhabit (Luo *et* *al.*, 2016). However, seawater have high salinity or concentration of mineral salts as high as 35g of salt in one liter of water (Bunnet, 1988; Mero, 2008).

In dry season, minerals in *L. harak* from the present study were higher than in wet season probably due to the fish species mode of living. It has been established that, the habitat of *L. harak* is usually in shallow sandy, mangroves, lagoons seagrass areas and lagoons (Aswani & Vaccaro, 2008). There was no dilution of seawater from rainwater during dry season. This could presumably cause the concentration of minerals to be more evident in fish by filter-feeding through swallowing of the seawater.

The results of the study were in agreement with the findings of Adelakun *et al.* (2017) who reported higher potassium, sodium and lower calcium contents in Catfish (*Clarias gariepinus*) during dry season. Although the sampling done by Adelakun *et al.* (2017) were from Jebba Upper Basin, the findings were still in the same opinion as the present study. The findings were not in agreement with those of Pal *et al.* (2017) who worked on Indian butter catfish (*Ompok bimaculatus*). In this study, the concentration of minerals were higher during in summer (wet season) than in dry season. This could possibly be induced by the water body content in Gomti river of Tripura where the fish were collected.

### 5.1.3 Mineral contents in *R. kanagurta*

The mineral contents in *R. kanagurta* were higher during dry season, however high contents of calcium were recorded intensive during wet season. Statistical test revealed a significant difference of mineral concentration between the seasons ($p < 0.05$) except in calcium content. Calcium contents being high in wet season may probably be induced by the feeding behavior of the fish species and temperature. In addition, the presence of  calcium than other minerals in rainwater (Ichikuni, 1978) may partly be affiliated with lower levels of mineral registered by this study. However, calcite being the source of calcium in rainwater comes from non-marine origins such as fertilizers or industrial wastes during rainwater flowing (Ichikuni, 1978). The presence of calcite in rainwater may probably cause an increase of calcium contents in *R. kanagurta* during rainy season. This report explained the phenomenon by noting that, the dilution occurs through flowing of water in rivers to the seawater bodies hence lowering the concentration level of minerals in fish. From the present study, it indicates a lower level of minerals in rainwater and slightly acidic content (Nelson, 2002) that may cause dilution of minerals during wet season.

Available research information shows that, the variation of fish composition depends on the quality of food available as fish feeds. This was demonstrated by Abdullahi, (2005) where the author attributed the variations being the function of nutrient availability and physiological state of the fish rather than the geographical location without marked limnological and climatic differences.

The results of this study in *R. kanagurta* shows a scenario that was similar to the findings reported by Paul *et al.*, (2015). It was further reported that, the minerals in Koi fish species *Anabas testudineus*, (Bioch, 1782) were higher in concentration during dry than wet season. Nevertheless, Khitouni *et al.* (2014) obtained results of potassium and calcium in *Diplodus annularis* (Linnaeus, 1758) were not in agreement with the present study. Researchers reported highest values of potassium and low calcium in wet season as opposed to what was reported in this study. The difference may presumably be caused by location differences (Khitouni *et al.* 2014) whereby the variation were associated with the fish feed and number of fish movement.

### 5.1.4 Mineral contents in *S. canaliculatus*

From this study, the concentration of potassium, sodium and magnesium in *S. canaliculatus* in wet season were lower than in dry season except in calcium content. However, the statistical analysis showed a significant difference of mean mineral contents in *S. canaliculatus* between the seasons ($p < 0.05$) except in calcium and magnesium. The high mineral existence in fish reported during dry season also concurred with the expression obtained by Shija *et al.* (2015) on mangroves. Authors attributed it by the low trophic level feeding on mangrove litter-falls therefore minerals were acquired by fish during feeding. The authors further noted that high content of minerals in litter-falls was found during dry season on the coastal zone. Considering *S. canaliculatus* habitat are in shallow waters in and around the mouths of rivers in turbulent waters (Westneat, 2006).

A study done by Balogun & Talabi (1986) who registered an elevated mineral contents in Skipjack tuna during wet rather than dry season differed from the current study. The researchers remarked that, the high presence of some minerals during wet season were caused by runoffs that carried dissolved minerals from adjacent land into the larger seawater and therefore made them readily available to the fish. Furthermore, the essential minerals were also in high demand for utilization during metabolic and other physiological processes. Paul *et al.* (2015) from India, investigated on Koi fish species *Anabas testudineus*, got rather similar results as those reported in this study. However, the observed variation of minerals may probably be caused by the changes in activities of the fish such as spawning and migration.

### 5.1.5 Effects of seasonal variability on mineral contents in fish species

From the results of this study, seasonal variability on the mean mineral contents from the four fish species (*A. ciliaris, L. harak, R. kanagurta, S. canaliculatus*) was statistically significant ($p < 0.05$) except in calcium. The mineral concentration of seawater depends on the surface and the amount of freshwater brought in by rivers (Bunnett, 2014). The changes of mineral contents could probably be explained by the differences of the fish species' ability to assimilate the feed (Prabhu, 2015) and the environmental condition like the coral reefs and mangroves, near river banks.

The temperature differences during the seasons (wet and dry) causes variation of mineral contents. It was known that water temperature plays an important role in influencing the fish feed consumption, metabolic rate and energy expenditure (Joshua *et al.,* 2017). The parameter affect the salinity, dissolved oxygen (DO) and concentration of seawater (Natarajan *et al.,* 2009). The result of evaporation of water in shallow coastal waters cause salinity to elevate (Edward & Head, 1987). Fluctuation of water temperature affects species like *R. kanagurta* that lives along the coast (Edward & Head, 1987).

## 5.2 Effects of Seasonal Changes on Proximate Contents in Fish Species

### 5.2.1 Proximate contents in *A. ciliaris*

The mean values of crude protein and lipids in *A. ciliaris* were high during wet season than in dry season (Figure 4.2a). Ash and moisture concentration were high during dry than in wet season. However, the variations of proximate contents in *A. ciliaris* between seasons were statistically significant ($p < 0.05$) except in ash. It has been reported that, differences in quality of the fish diet presumably causes variation in the fish body constituents (Ayuba & Iorkohol, 2013). In wet season, presence of nutrients may be included as the runoffs that are carried into the seawater and therefore made them readily available to the fish (Abdulkarim *et al.*, 2016). Abundance of food supply can markedly change the body composition of the species.

The results of the present study concur with the results done by Olgunoglu *et al.* (2014) in Mesopotamian Catfish (*Silurus triostegus*) on lipid, ash and moisture contents. Boran & Karacam, (2011) who studied on fish horse mackerel found similar results to the study in crude protein and lipids. The scenario was in contrast with findings of ash and moisture by Saoud *et al.* (2008) in Lebanon analyzing *Siganus rivulatus* and *Diplodus sargus*.

The results of moisture and lipids of the current study were contrary to those found by Bandarra *et al.* (2001) who worked with horse mackerel (*Trachurus trachurus*) collected from Portugal. Lipid contents in *A. ciliaris* from this study were higher in wet than in dry season. In Turkey, Kaçar *et al.* (2016) studied on *Silurus triostegus* and reported a contrasting results in which lipid concentration were higher in dry season similar to those documented by Nisa & Asadullah (2011). In the present study, the results of moisture contents were higher in dry than wet season different from those reported by Nisa & Asadullah (2011).

In this study, the concentration of ash were higher (Figure. 4.2a) during dry season. It was proved that, variation of nutrients may appear due to the difference from one fishing ground to another that influence the fish composition (Deka *et al.,* 2012). This may be due to the amount, quality of the fish feed and mobilization of nutrients in the fish body system. These findings agrees with those remarked by Adelakun *et al.*,(2017).

Results documented by Nargis (2006) who studied *Anabas testudineus* from Bangladesh did not conform with this study. The contrasting results may perhaps be explained by location differences as reported by Bunnet (1988). Despite of that, the divergence of the findings were apparently re-presenting physiological changes as fishes had different spawning periods and sizes (Cui & Wootton, 1988; Erisman *et al.,* 2011). It has been reported that, differences in quality of the fish diet presumably causes variation in the fish body constituents (Ayuba & Iorkohol, 2013). It was recorded by Bagthasingh *et al.* (2016) that, proximate composition of fish are closely related to the feed intake and the variation in the lipid content are much wider than that in crude protein.

### 5.2.2 Proximate contents in *L. harak*

The mean proximate composition in tissues of *L. harak* showed that, crude protein and lipids concentration were higher in wet season while ash and moisture contents were lower. However, t-test showed a statistical significant difference of lipids and moisture contents in tissues of *L. harak* between seasons ($p > 0.05$) except in ash and crude protein contents. Variation of proximate concentrations between the seasons may apparently be influenced by eutrophic waters caused by seasonal nature of food input in the fish environment (Bailey & Robison, 1986). Moisture content being high during dry season could presumably be due to physiological attempt of fish bodies to neutralize the concentration of minerals in the surrounding water due to little amount of water for dilution (Bagthasingh *et al.,* 2016). The variation may probably be caused by species differences as observed in the study of Debnath *et al.,* (2014).

From the present study, the difference of proximate contents between seasons are in conformity with the report of Bagthasingh *et al.* (2016) from Thoothukudi coast in India. Other researchers Küçükgülmez *et al.* (2008) in Turkey also found similar results of ash, lipids and moisture contents. The results of the present study in wet season showed a higher concentration of protein and lipids and lower moisture contents. These results concurred with those documented by Boran & Karacam, (2011) in both horse mackerel and shad. In the study of Kiran *et al.* (2017) the results of moisture, protein and lipids during dry season were similar to those reported in the present study. The slightly raising of ash contents reported in this study concur with the experimental results reported by Olgunoglu *et al.,* (2014). Contrasting findings of ash, lipids and moisture contents from the present study were experienced in Nigeria by Effiong & Mohammed (2008). There was also a divergence of results in a study done by Hussain *et al*. (2016) in Pakistan on carnivorous fish.

### 5.2.3 Proximate contents in *R. kanagurta*

Results from the study showed that crude protein and lipids concentration in tissues of *R. kanagurta* were higher during wet than dry season while ash and moisture contents were lower in wet than dry season. The statistical test showed no significant difference of proximate contents in *R. kanagurta* between the seasons ($p > 0.05$) except in lipids. With an exception of lipid findings, the results of the proximate contents from the current study were in agreement with the results of Azim *et al.* (2012) from Bangladesh who studied *S. panijus.* The difference of results of lipid could be caused by variation in seawater temperature. From the present study, *R. kanagurta* collected during wet season had higher lipid contents therefore the fish species tend to balance the body temperature with the environment.

The results of the study done by Olgunoglu *et al.* (2014) from Turkey in *S. triostegus* were similar to those recorded in the present study. Those results may apparently be caused by *S. triostegus* being at spawning period between May and late June (Oymak *et al.* 2001) causing high protein. The results of protein contents in the present study during wet season may probably be synthesized by the body used in maturation and production of eggs (Rodríguez-González *et al.*, 2006). In addition, the fish species in this study is a carnivorous fish (Kaçar *et al.*, 2016).

The findings of *Mullus barbatus* and *Merluccius merluccius* experimented by Tulgar & Berik (2012) varied whereby only *Merluccius merluccius* agreed with those found in the current study. Similar results of proximate concentration in tissues of *R. kanagurta* from the current study were reported by Boran & Karacam, (2011); Khitouni *et al.* (2014); Rani *et al.*, (2016). It was reported by Sargent *et al.* (1995) that, the species difference and its habitat affects the natural diet of the fish that influence the nutritional composition of the fish. However, the results of the proximate contents in the current research, disagreed with the findings of *Anabas testudineu*s done by Paul *et al.*, (2015). The difference may presumably be generated by the fish species' habitat where *R. kanagurta* were collected in Indian Ocean while *Anabas testudineu*s are feshwater fish species. It was remarked that, water temperature fluctuation affect fish growth rate (Jawad, 2001). Nevertheless, smaller size of fish results to higher moisture content and lower protein content (Da Silva *et al.* 2008) this may cause variation of proximate contents between species.

In the current study, the results of moisture content were higher in dry than wet season different from those reported by Nisa & Asadullah (2011). Despite of the researchers experimenting the same species with the present study, the reported result of moisture content was different. This difference would presumably be due to individual fish species or the analyzed portion of the sample as proclaimed by Torry (2018). The researcher reported the different nutrient contents depended on the collected portion of the fish muscle.

### 5.2.4 Proximate contents in *S. canaliculatus*

The contents of protein and lipid in tissues of *S. canaliculatus* were higher in wet than in dry season, while ash and moisture contents were lower. The difference of proximate contents in *S. canaliculatus* was significant between seasons ($p < 0.05$) except in ash. Similar findings were obtained by Boran & Karacam, (2011) and Bagthasingh *et al.* (2016) where the concentration of crude protein and lipid were high in wet season. This scenario could be associated with the feeding habit omnivorous/herbivorous of the fish species and the ability to feed low trophic level on the aquatic chain (Tacon *et al.*, 1990). The results of protein, ash and moisture in both species *Euthynnus affinis* and *Auxis thazard* (Rani *et al.* 2016) conform with those in *S. canaliculatus* from the present study. The similarity could possibly be as a result of the species belonging to the same family Scrombidae, therefore had matching characteristics.

In this current study, lipid content was high during wet season. Similar findings were obtained and reported in the study done by Adelakun *et al.* (2017) where lipid contents were higher during rainy season. The results are also in agreement with the findings of Kaçar *et al.* (2016) in *S. triostegus* from Turkey. The present results agree with the study of Bandarra *et al.* (2001) where the variation of lipid and moisture contents showed similarities. However, the study conducted by Saoud *et al.* (2008) on *Siganus rivulatus* depicted opposing results despite the fact that the studied species *Siganus canaliculatus* belongs to the same genus. The situation could apparently be caused by variation of body weight of the fish species and regional differences (Bailey & Robison, 1986). The location of the present study is neither in Oligotrophic nor Eutotrophic zone (Polovina *et al.* 2008) while location used by Saoud *et al.* (2008) was in Eutotrophic region. The findings of Paul *et al.* (2015) in *Anabas testudineus* from India disagreed with the variation of proximate contents between seasons in the present study.

Similar scenario to the present study in ash contents during dry season were experienced and documented by Olgunoglu *et al.* (2014) and Adelakun *et al.*, (2017). A deviation of results was noted in the study done by Ashwini *et al.* (2016) in male *D.*

*russelli* from India. The deviation of the registered phenomenon from the present study may probably be induced by variation of fish catch environment (Ashwini *et al.,* 2016). In the present study, ash content were high in dry season. The study of Nargis (2006) and Effiong & Mohammed (2008) had contrary findings of ash content in wet season different from the present study. The difference may apparently be induced by the seawater fish of the present study and freshwater fish. Ash being a measure of total amount of minerals in fish (Pomeranz & Meloan, 1994b) was high in dry season presumably due to balancing with the seawater environment from the fish body.

Moisture content in *S. canaliculatus* was low during wet than dry season. The findings disagreed with the report of Adelakun *et al.* (2017) whereby moisture content in wet season was higher than in dry season. The variation of proximate contents may possibly be caused by spawning period of fish species occurs in May and June while the studied fish *S. canaliculatus* were collected in early April. This was just before the start of spawning thus the protein content in fish were high in wet season due to heavy feeding (Boran & Karacam, 2011).

### 5.2.5 Effects of seasonal variability on proximate contents in fish species

From the results of this study, seasonal variation in proximate contents from the four fish species (*A. ciliaris, L. harak, R. kanagurta, S. canaliculatus*) are shown in Table 4.3. The variation of proximate contents in all fish species were statistically between seasons ($p < 0.05$) except ash in all fish species, crude protein in *L. harak* and *R. kanagurta* and moisture in *R. kanagurta*.

It was known that, the habitat of *R. kanagurta* and *S. canaliculatus* are pelagic and shallow water respectively and are both omnivores. The variation of chemical composition of fish varies greatly with species, sex, age, environment and season (Alemu *et al.* 2013; Sonavane *et al.,* 2017). Temperature differences caused as a result of seasonal variability may apparently affect the fish feed consumption, metabolic rate and energy expenditure.

During wet season, the variation of nutrients may probably be due to flowing water exchange and high flowing of nutrients (Blé & Arfi, 2009). Abundance of food supply can clearly change the biochemical composition of fish species while overcrowding may cause insufficiency of food resulting to variation of their body composition (Deka *et al.*, 2012). Spawning process of the fish species may be at same locations at either simultaneously or at different times of day, month or year, while other locations may be occupied by only a single species (Erisman *et al.,* 2011).

## 5.3 Effects of Processing Methods on Minerals in Fish Species

### 5.3.1 Effects of processing methods in *A. ciliaris*

The results of this study showed slight variation in mineral contents between raw and fried tissues of *A. ciliaris.* However, the difference of mineral contents in fried *A. ciliaris* compared raw were insignificant ($p > 0.05$). The decrease of mineral contents in fried fish were supported by the study done by Steiner-Asiedu *et al.,* (1991). The researchers reported that, tilapia (*Tilapia sp*) a freshwater fish and marine flat sardine (*Sardinella sp*) and sea bream (*Dentex sp*.) showed a decrease of the minerals concentration when fried except for sodium, calcium and magnesium in Sea bream and sodium in Tilapia. Although flat sardine and tilapia fish species were collected from different aquatic environments, their minerals' characteristics were similar probably due to their high proportion of bones (Steiner-Asiedu *et al.,* 1991).

The study of Marimuthu *et al.* (2014) bears some results similar to the analyzed minerals in the current study such as magnesium and calcium contents reduced in fried fish compared to raw fish. The results of sodium and potassium contents in the study of Marimuthu *et al.* (2014); Karimian-khosroshahi *et al.* (2016) were not in conformity with those reported in the present study. They had an increase of mineral content upon frying that may probably be caused by the differences of the fish species that have different muscle capabilities to retain or release biochemical nutrients (Ikanone & Oyekan, 2014). The results of Steiner-Asiedu *et al.* (1991) who studied flat sardine (*Sardinella* sp.) found the same trend of results as the current study. The findings of fried tilapia were in line with results reported from this study except in sodium which showed an increase in concentration.

Results of raw compared to boiled tissues of *A. ciliaris* showed slight decrease of mineral contents except in calcium, however the difference of mineral contents were statistical insignificant ($p > 0.05$). The composition of the medium used for boiling *A. ciliaris* may affect the concentration of minerals. It was known that, the presence of mineral such as major ions, minor ions, nutrients and trace elements may affect the water quality (Health, 2014). Leaching out of minerals due to breakage or bonding of ions during boiling may cause the presence of minerals to increase or decrease (Bethke & Jansky, 2008).

The results of potassium and sodium concentration in the current study were in agreement with the recorded results of the study done by Karimian-khosroshahi *et al.* (2016) in boiled rainbow trout (*Oncorhynchus mykiss*) from Iran. The present results disagreed with those reported by Marimuthu *et al.* (2012) who worked with striped snakehead fish (*Channa striatus*).

### 5.3.2 Effects of processing methods in *L. harak*

From the present study, the minerals in fried *L. harak* were lower in comparison to raw fish except calcium, however the difference of minerals between raw and fried tissues of *L. harak* were insignificant ($p > 0.05$). The results of the current study comply with those reported by Hosseini *et al.* (2014) who experimented in kutum roach (*Rutilus frisii kutum*) except potassium content that increased. The study of Karimian-khosroshahi *et al.* (2016) in rainbow trout (*Oncorhynchus mykiss*) disagreed with the findings of the present work.

Despite *Rutilus frisii kutum* in the former and *O. mykiss* in the latter studies being collected in the same region yet, the findings were controversial. The difference of mineral values was seemingly due to water holding capacity of the analyzed tissues (Ofstad *et al.* 1993) that differs between one individual fish to the other. The similarities of results of Hosseini *et al.* (2014) and the difference in the findings of Karimian-khosroshahi *et al.* (2016) from the present study may presumably be generated by the difference of fish species. Deviation of results were reported in the study performed by Gokoglu *et al.* (2004) where fried rainbow trout had higher sodium and potassium and lower calcium contents in comparison to raw fish.

The minerals in raw fish species from the current study were higher than in boiled fish except in calcium. The t-test indicated no statistical significant difference of mineral contents between raw and boiled *L. harak* ($p > 0.05$). A recent study of Cristelle *et al.* (2018) done in Cameroon, reported similar findings of decrease of mineral contents from four analyzed fish species. Similar results of sodium, potassium contents to the current work were observed in the experiment of boiled *Oncorhynchus mykiss* done by Karimian-khosroshahi *et al.,* (2016). Moreover, the result of minerals in boiled rainbow trout by Gokoglu *et al.* (2004) were in agreement with those of the current work except in calcium contents. This difference of results may probably be caused by time, temperature and water constituents used for boiling the fish as earlier echoed by Severi *et al.,* (1997).

The findings of the minerals obtained by Karimian-khosroshahi *et al.* (2016) in boiled rainbow trout from Iran, were in conformity with the present study except magnesium. However, the results of the study recorded by Gall *et al.* (1983) disagreed with the present study. Among the fish species experimented by Gall *et al.* (1983), was the red snapper belonging to the same family with *L. harak* of the present study, yet the results did not match with those in the present study. The species differences may apparently be the source of deviation of the findings where the mineral loss ability during cooking differs. It may also possibly be affected by the duration and medium used for cooking (Cui & Wootton, 1988).

### 5.3.3 Effects of processing methods in *R. kanagurta*

In comparison to raw *R. kanagurta*, a decrease of the analyzed mineral contents in fried fish were registered in the current study. The statistical t-test showed insignificant difference of mineral contents from tissues of raw *R. kanagurta* compared to fried ($p > 0.05$). The decrease of minerals in fried fish presumably be induced by the holding capacity of the fish muscles to retain minerals during frying (Ofstad *et al.*, 1993). Findings reported by Steiner-Asiedu *et al.* (1991) were similar in *Sardinella sp.* while sea bream had contradicting results of sodium, magnesium and calcium contents from the present study.

The research findings of Kapute & Sainani (2017) in *O. karongae* from Malawi were in agreement with the current work with regards to the contents of calcium and magnesium. Nevertheless, according to Marimuthu *et al.* (2012), the findings of sodium, potassium and magnesium contents disagreed with the present study. The report from Mustafa & Medeiros, (1985) also differed with the findings of the current study in sodium, magnesium and calcium contents of catfish (*Ictalurus punctatus*).

In boiled *R. kanagurta*, mineral contents showed a decrease when compared to raw fish except in calcium although the difference was insignificant ($p > 0.05$). The decrease of minerals contents in fish when boiled may apparently be caused by leaching of minerals from fish tissues into the boiling medium (Bethke & Jansky, 2008).

The results of the study were in agreement with those reported by Gokoglu *et al.* (2004); Lates *et al.* (2014) in all minerals. The report of calcium and magnesium contents in the studies of Karimian-khosroshahi *et al.* (2016); Hosseini *et al.* (2014) deviated from the results stated in the present study. This variation may apparently be caused by loss or structural destruction that occurs during boiling (Harris, 1988). It may permeates in fish tissues and the contained compound in the cooking medium becomes soluble materials and leach out to the cooking medium.

The researchers Steiner-Asiedu *et al.* (1991) studied flat sardines and reported results that corresponded with the present study. The results of calcium and magnesium in the study done by Kapute & Sainani (2017) were in agreement with the recorded findings of the present study. Other scientists that recorded contrary results from the current study were Karimian-khosroshahi *et al.,* (2016). The results of the other studies deviating from the current study may probably be induced by the duration time used for cooking which was reported as a factor that influences the quality of the end-product of the fish biochemical composition (Farag, 2013).

### 5.3.4 Effects of processing methods in *S. canaliculatus*

From the current study, potassium, sodium and magnesium decreased in contents when analyzed in fried tissues of *S. canaliculatus* in comparison to raw fish except in calcium. The t-test showed insignificant difference of minerals in raw *S. canaliculatus* when compared to fried ($p > 0.05$).

The trend of calcium and magnesium content in the studies of Gall *et al.* (1983) and Mustafa & Medeiros (1985) were similar to the present study except in potassium and sodium. Contrary results from the current study were experienced in the researches of Marimuthu *et al.* (2012) and Marimuthu *et al.*, (2014). The observed variation of results may be induced by the difference of the quality of cooking oil used during frying. Cooking oils may have different contents and thus affect the end product fish body composition (Weber *et al.*, 2008).

The outcome of potassium sodium and magnesium obtained in boiled tissues of *S. canaliculatus* were lower compared to those recorded in raw fish. The t-test proclaim no statistical significant difference of mineral contents between raw and boiled *S. canaliculatus* ($p > 0.05$). The decrease of minerals when the fish tissues were processed may be influenced by heat that break the bonds causing loose minerals to leach out into the cooking medium (Karimian-khosroshahi *et al.,* 2016).

Comparable results of sodium and calcium in fish of the present study agreed with the results of the study of Hosseini *et al.* (2014) and Karimian-khosroshahi *et al.,* (2016). The findings of sodium and potassium reported by Gokoglu *et al.* (2004) agreed with the results of the present study. Different findings were reported from the study done by Marimuthu *et al.,* (2012).

### 5.3.5 Effects in minerals due to processing methods

The present study showed mineral contents in the fish species decreased when processing methods were applied except in calcium. However, t-test showed no statistical difference of mineral contents between raw and fried, raw and boiled and fried and boiled ($p > 0.05$) with an exception of potassium in boiled fish. Minerals leach out into the cooking medium and may be damage or changed and cause distortion on the chemical composition (Karimian-khosroshahi *et al.*, 2016). Earlier researchers Gall *et al.* (1983); Steiner-asiedu *et al.* (1991) got similar results of little or no effect of processing methods in minerals. However some reports showed variation in some fish species that were affected by the processing methods (Marimuthu *et al.* 2012; Hosseini *et al.,* 2014). Moreover, during fish processing by heat, mineral solubilisation may occur (Bastías *et al.,* 2017).

## 5.4 Effects of Processing Methods on Proximate Contents in Fish Species

### 5.4.1 Effects of processing methods in *A. ciliaris*

Proximate composition in fried *A. ciliaris* showed an increase of ash, protein and lipid contents coupled with a decrease of moisture contents. The variation of proximate contents in *A. ciliaris* was significant ($p < 0.05$) except in ash content. During frying as a drying process of the fish samples, moisture contents from the fish samples was converted in the form of vapor and frying oil was absorbed simultaneously (Fan *et al.*, 2005). The removal of water in fish samples exposes other nutrients such as protein and ash. The results of the study are in agreement with those done by Ersoy & Özeren, (2009). The study of Mustafa & Medeiros (1985) was fairly in-line with the present work in findings of moisture and ash.

In the present study, the changes that occurred in moisture were supported by Rahman *et al.* (2012) and those of protein were in accord with results reported by Ayinsa & Maalekuu (2013). The changes of proximate contents in fried *A. ciliaris* could probably be induced by duration of frying that may cause an increase or decrease of nutrients and also nature of fish feed ingested.

Upon boiling of *A. ciliaris* tissues, there was an increase of protein and lipid contents with a decrease of moisture and ash contents in fish. The difference of ash and moisture in boiled fish compared to raw was statistically insignificant ($p > 0.05$) except in crude protein and lipids. Similar results to the present study were reported by Asghari *et al.* (2013) where there was a declined of moisture and ash contents and a raised levels of protein and fats contents. The authors Marimuthu *et al.* (2014) evaluated *L. calcarifer* and reported findings that were not in accord with the present study for attributed of proximate composition of the fish samples. The trend of results in boiled fish from the present study were similar to those reported by Aberoumand (2014) in only moisture. The difference of results may presumably be induced by the difference of environmental regions.

The present study yielded results which were similar to the findings reported by Mustafa & Medeiros, (1985) in catfish collected from Mississippi and Ansorena *et al.* (2010) in *Gadus morhua* and *Salmo salar* from Spain. Opposed findings from the present study were observed in boiled fish that had lower lipid content and higher ash content than in raw fish. The results of Marimuthu *et al.* (2012) who studied striped snakehead fish (*Channa striatus*) agreed with the present work except in results of ash in boiled fish. The registered variation could probably be attributed by the effect of fish age and the stage of life cycle. As it has been established that, starvation of fish may cause a decrease of protein and lipid (Boran & Karacam, 2011) and the weight of the fish as stated by Kasozi *et al.*, (2014).

### 5.4.2 Effects of processing methods in *L. harak*

The findings of proximate contents in fried tissues of *L. harak* showed a decrease in ash and moisture and increase of protein and lipids. However, statistical test has revealed a significant difference between raw and fried ($p < 0.05$) except in ash content. It was known that, frying fish reduces the tissue platelet aggregation capacity (Ansorena *et al.* 2010) and resulting to moisture turning to vapor thus moisture content reduces in fish tissues. Frying causes nutrient bioavailability (Ikanone & Oyekan, 2014) and lipid content increased due to absorption of oil in fish tissues during frying. Nevertherless, the results of all the analyzed constituents were in agreement with the study done by Kapute & Sainani (2017) except where authors reported a decrease in protein content.

Findings of Aberoumand, (2014), tend to agree with the results registered in the present study with regards to moisture and lipids contents. The findings of ash and protein contents in the present study were not in accord with the report of Aberoumand & Ziaei-Nejad (2015) as well as results of ash in the study done by Gall *et al.* (1983) and Marimuthu *et al.,* (2012). Deviation of ash contents in the current study from other studies may probably be induced by the duration time used during frying.

During cooking, chemical and physical reaction takes place and causes loss of water and increase of lipid contents (Afolayan, 2015) due to absorption of the cooking oil as observed in the present study. Similar findings were reported in the studies of Marimuthu *et al.* (2012); Karimian-khosroshahi *et al.* (2016) with slight deviation in the result of ash contents. According to the findings of Kapute & Sainani (2017), the value of protein in fried *Oreochromis karongae* did not comply with the current work.

In boiled *L. harak,* ash and moisture contents were lower than in raw fish, while protein and lipid values were higher than in raw fish. Yet, the t-test indicated an insignificant difference of proximate contents in boiled *L. harak* compared to raw fish ($p > 0.05$) except in lipid contents. Similar results were noted in the study of Huque *et al.* (2014) who worked in *Pampus argenteus*. Contrary findings of lipids contents were registered in the study of Cristelle *et al.,* (2018).

Findings of boiled rainbow trout in the research done by Karimian-khosroshahi *et al.* (2016) and Catfish that was experimented by Mustafa & Medeiros (1985) disagreed in ash where the contents increased. The deviation of results in ash contents from other authors to the present study may possibly be attributed to the time used for boiling. This is quite often gets extended to allow water to permeate in fish muscles and the compounds become rather soluble materials (Cristelle *et al.,* 2018). The researchers who worked in *Rutilus frisii kutum* (Hosseini *et al.* 2014) acquired results that were not in accord to the results of the present study. The disparity of results from the current study may probably be induced by the medium of cooking that was known to have an impact to the end products (Gall *et al.,* 1983).

### 5.4.3 Effects of processing methods in *R. kanagurta*

In fried *R. kanagurta,* ash and moisture contents were lower as compared to raw fish while protein and lipid contents were higher than in raw fish. Analysis test showed statistical significant difference of proximate contents in fried tissues of *R. kanagurta* compared to raw fish ($p < 0.05$). Ash was defined as total amount of minerals in fish and have low volatility as compared to other components (Pomeranz & Meloan, 1994). However during frying, water molecules in fish tissues turns to vapor thus dry matter was obtained and other components were revealed. Therefore, there was a loss of moisture, exposing the protein components and also oil penetration in fish tissues during cooking (Saguy & Dana, 2003).

Similar changes of proximate contents were reported by Hosseini *et al.* (2014) in the study of *Kutum roach* from Iran except ash contents. Comparable scenario was experienced by Gokoglu *et al.* (2004); Karimian-khosroshahi *et al.,* (2016). The results of the study of Steiner-Asiedu *et al.* (1991) unveiled contrasting results of protein contents compared to the present study. The outcome of the present study were also reported by Gokoglu *et al.* (2004) and Weber *et al.* (2008) with an exception of ash mean content results. Contrary findings from the present study were reported by Marimuthu *et al.* (2014) except in protein mean contents that were similar to the present study.

The findings of proximate contents in boiled *R. kanagurta* compared to raw fish showed a decrease of moisture and ash contents and an elevated values of protein and lipids. The statistical test indicated no significant variation of proximate contents in boiled tissues of *R. kanagurta* compared to raw ($p > 0.05$). The decrease of moisture and ash contents in boiled fish were seemingly influenced by evaporation of moisture during boiling causing protein and fat contents to increase significantly (García-Arias *et al.,* 2003). The findings reported by Huque *et al.* (2014) corresponded with the results of the present study. Other researchers Gokoglu *et al.* (2004); Bassey *et al.* (2014) reported results that were in consensus with the current study except ash contents that were higher in boiled fish than raw. The results of proximate contents in boiled *Lates calcarifer* when compared to raw that was experimented by Marimuthu *et al.* (2014) disagreed with the report of the present work. The variation of results of the present study may possibly be generated by the species differences and ability of fish muscles to retain or release the proximate constituents (Farag, 2013).

### 5.4.4 Effects of processing methods in *S. canaliculatus*

Findings of the present study unveil a decrease of ash and moisture contents while protein and lipids showed an increased content in fried tissues of *S. canaliculatus* compared to raw fish. A statistical test indicated significant variation of proximate contents in fried *S. canaliculatus* in comparison to raw ($p < 0.05$). The inclination of the findings in the present study were presumably due to water losses, that occurred during frying and resulted to higher protein content in fried fish compared to raw (Marimuthu *et al.*, 2012).

Corresponding results from the current study was acquired by Farag (2013) and to some extent Weber *et al.* (2008) and Ansorena *et al.* (2010) except ash contents that was higher than in raw fish unlike the results of the present study. Moreover, the study done by Aberoumand & Ziaei-Nejad (2015) experienced different results of protein and ash contents in comparison to the current work. The findings reported by Kapute & Sainani (2017) disagreed with the findings of the current study displayed in protein contents. The variation of proximate composition may perhaps be as a result of the duration of frying the fish and the difference of fish muscles' ability to absorb oil and volatility of the fish constituents. It was explained by Frankel (1991) that decrease of moisture during cooking assist in preservation of nutrients by reducing the fish susceptibility to microbial spoilage, oxidative degradation of poly unsaturated fatty acid. The end product of various cooking oils causes difference of nutrients after frying (Omotosho *et al.*, 2015).

The reported results of proximate contents in the current work showed a decrease of ash and moisture contents in boiled *S. canaliculatus* when compared to raw fish. Protein and lipids in boiled *S. canaliculatus* were higher in contents in comparison to raw fish. A t-test of significance indicated no difference of proximate contents in boiled *S. canaliculatus* compared to raw ($p < 0.05$) except in lipids.

The results of the present work were in agreement with the report of Karimian-khosroshahi *et al.* (2016) except that of ash contents. Out of four species in the study done by Cristelle *et al.* (2018) the outcomes of moisture contents in all species and ash in *Cyprinus carpio* concurred with results of the present study. Protein, lipids and ash contents in the research of Cristelle *et al.* (2018) disagreed with the current work. Incompatible findings from the current study were also observed by study of Kapute & Sainani (2017) in protein and fats contents. The observed variation of results in boiled *S. canaliculatus* may apparently be brought about by the species differences as it has been recently remarked in the findings of the study by Cristelle *et al.*, (2018).

Similar results were recorded by Gokoglu *et al.* (2004) and Marimuthu *et al.* (2012) except ash contents that increased in processed fish. Results reported by Marimuthu *et al.* (2014) were in conformity with the present work on means proximate contents when boiled.

### 5.4.5 Effects in proximate contents due to processing methods

The results of proximate composition of the current study showed a decrease of ash and moisture in fried compared to raw fish. Lipid contents in boiled fish compared to raw, was higher while other components decreased. However, there was no significant difference of ash content in raw compared to boiled fish and fried fish also boiled compared to fried, similarly crude protein and moisture between raw and boiled fish ($p > 0.05$). The decrease of ash contents in processed fish species compared to raw, agreed with the decrease of minerals in processed fish species. This can be explained by considering ash as the total amount of minerals present in fish muscles (Pomeranz & Meloan, 1994b). The results of proximate contents in processed fish species were in agreement with the studies of Karimian-khosroshahi *et al.* (2016) and Cristelle *et al.*, (2018).

### 5.5 Predicted values of Mineral and Proximate Contents due to seasons

The predictive model values showed unique higher contribution of season in potassium, sodium and magnesium contents and little contribution to calcium contents. This results indicated that season was the main cause of variation of potassium, sodium and magnesium in tissues of the analyzed fish species. As a result, the predicted model generated realistic estimates of changes in the mineral contents. The implication is that season is not the main cause of variation of calcium contents. There may be other factors that affect Calcium such as calcite in rain water, (Ichikuni, 1978) and dynamic processing of sediments (Encyclopedia, 2018).

On the other hand, changing seasons had little influence on proximate contents. This could probably be due to bio-energetic process that includes fish feed, geographical location and growth pattern of the fish species (Deangelis *et al.* 1993; Vollenweider *et al.*, 2011).

### 5.6 Predicted Values in Mineral and Proximate Composition due to processing methods

The model has indicated a decrease of potassium, sodium and magnesium contents when processed. This could probably be explained by the reactivity of the minerals (Lenntech, 1998). During cooking, time and temperature had an effect on nutrient loss whereas the longer the cooking time, the higher the nutrient losses (Kristy &Allison, 2007).

The water loss and absorption of fats due to processing methods results in decrease of ash and moisture contents and increase of protein and lipid in the fish species. A study done by Adelakun *et al.* (2017) has indicated that moisture and lipid contents are inversely proportional to each other. That is, a decrease in moisture will cause an increase in lipid contents. This finding is in agreement with those reported by García-Arias *et al.* (2003) where proximate contents were high for fried fish while ash contents decreased. The chemical and physical reactions occurring during processing may either improve or impair the biochemical composition of the fish (Bognár, 1998). However, processing of fish should both maximize nutritional benefit as well as minimize the potential negative effects.

## 5.7 Principal Component Analysis

There was a strong correlation between seasons and minerals. Seasonal variation had an effect in the determined minerals. Seawater contains minerals such as potassium and sodium. During wet season dilution due to rainwater probably reduces the concentration of the minerals in seawater. Variation of calcium content may be caused by other sources since seasonal variation had only a little effect.

Little correlation of proximate contents with seasonal variation indicated that variation of proximate contents are not directly affected by seasons. Protein and lipid contents, on the other hand, had a strong correlation indicating their relationship. There was a strong correlation of processing methods to ash contents indicating the effect of processing methods on ash content.

# CHAPTER SIX

# CONCLUSION AND RECOMMENDATIONS

## 6.1 Conclusion

The findings of the study have shown that, mineral contents of selected fish may vary due to seasons. Changing of seasons did affect the mineral contents in all the selected fish species and the effect was similar in potassium, sodium and magnesium minerals. Mineral contents did not change significantly due to the applied processing methods. The variation of mineral contents due to changing of seasons were accurately predicted by the derived model. Furthermore, the derived model predicted the variation of potassium content only when processing methods were the predictor, indicating that the processing methods have insignificant effect on the variation of the other minerals.

Proximate contents in studied fish species also varied with varying seasons. Whereas protein and lipid contents increased in wet season, moisture and ash contents increased in dry season. In addition, the effect of boiling processing method to proximate contents in fish was lower than the frying processing method. The variation of proximate contents due to changing of seasons were accurately predicted by the derived model. In addition, variation of ash contents was accurately predicted by the model in both processing methods. However, the model failed to predict variation of lipid, protein and moisture when using boiling processing method.

## 6.2 Recommendations

Recommendations from this study, will be as follows:

- Encourage the community on fish consumption due to the presence of potential fish biochemical components in both seasons.

- Since the essential nutrients such as protein, fatty acids and essential minerals that are necessary to human health are less affected in boiled fish compared to fried fish, more emphasis on boiled fish consumption to the community should be done.

## 6.3 Implication for future research

Further research is required for better understanding on other factors that influenced the variation of proximate contents and calcium minerals besides seasonal variation. There is a need to investigate the sources of variation of mineral contents in processed fish species as well. In addition, more studies are needed to derive a model that can predict variation of minerals due to both frying and boiling processing methods. Moreover, there is a need to derive a model that can accurately predict the variation of proximate contents (protein, lipid, and moisture) due to boiling processing method as a predictor. Further research can also be done on the variation of mineral and proximate contents due to other processing methods such as steaming and microwaving.

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# APPENDICES

## 1. *Alectis ciliaris*

| | Wet | Dry | F value | p value |
|---|---|---|---|---|
| **Potassium** | 0.29±0.13 | 8.15±3.42 | 14.10 | 0.00 |
| **Sodium** | 0.57±0.32 | 6.02±3.20 | 39.42 | 0.00 |
| **Calcium** | 0.22±0.13 | 0.15±0.07 | 0.74 | 0.40 |
| **Magnesium** | 0.16±0.08 | 0.76±0.39 | 3.72 | 0.07 |

| | Wet | Dry | F value | p value |
|---|---|---|---|---|
| **Ash** | 10.05±3.71 | 13.88±5.48 | 0.22 | 0.88 |
| **Protein** | 16.51±7.37 | 8.62±3.60 | 18.85 | 0.00 |
| **Lipid** | 5.1±1.37 | 1.64±0.83 | 23.27 | 0.00 |
| **Moisture** | 58.18±14.23 | 69.85±11.99 | 19.23 | 0.00 |

| | Potassium | Sodium | Calcium | Magnesium |
|---|---|---|---|---|
| **Raw** | 5.36±2.40 | 4.37±1.11 | 0.18±0.05 | 0.60±0.26 |
| **Fried** | 4.46±1.89 | 3.64±1.22 | 0.18±0.07 | 0.42±0.29 |
| **Boiled** | 3.05±1.28 | 2.84±0.89 | 0.19±0.06 | 0.47±0.15 |
| **F value** | 0.47 | 0.29 | 0.02 | 0.32 |
| **p value** | 0.63 | 0.75 | 0.98 | 0.73 |

| | Ash | Protein | Lipid | Moisture |
|---|---|---|---|---|
| **Raw** | 13.05±3.87 | 6.15±1.37 | 0.20±0.08 | 74.45±3.96 |
| **Fried** | 14.25±3.57 | 23.37±5.73 | 9.36±1.45 | 45.68±14.66 |
| **Boiled** | 8.59±2.98 | 8.19±2.08 | 0.55±0.19 | 71.91±5.48 |
| **F value** | 1.68 | 15.96 | 18.03 | 18.64 |
| **p value** | 0.19 | 0.00 | 0.00 | 0.00 |

## 2. *Lethrinus harak*

| | Wet | Dry | F value | p value |
|---|---|---|---|---|
| **Potassium** | 0.32±0.09 | 8.56±2.82 | 16.524 | 0.00 |
| **Sodium** | 0.54±0.24 | 5.72±2.35 | 22.858 | 0.00 |
| **Calcium** | 0.23±0.11 | 0.12±0.06 | 1.675 | 0.21 |
| **Magnesium** | 0.19±0.04 | 0.65±0.18 | 31.926 | 0.00 |

| | Wet | Dry | F value | p value |
|---|---|---|---|---|
| **Ash** | 8.62±3.56 | 11.08±3.99 | 0.04 | 0.84 |
| **Protein** | 14.93±7.31 | 12.07±5.26 | 2.14 | 0.15 |
| **Lipid** | 5.84±3.85 | 2.81±0.79 | 11.02 | 0.00 |
| **Moisture** | 59.62±15.61 | 66.55±11.84 | 7.62 | 0.01 |

| | Potassium | Sodium | Calcium | Magnesium |
|---|---|---|---|---|
| **Raw** | 6.29±2.78 | 3.90±1.66 | 0.11±0.07 | 0.46±0.18 |
| **Fried** | 4.71±1.78 | 3.08±1.17 | 0.20±0.08 | 0.39±0.18 |
| **Boiled** | 3.08±1.35 | 2.81±0.95 | 0.20±0.05 | 0.44±0.20 |
| **F value** | 0.88 | 0.22 | 1.85 | 0.12 |
| **p value** | 0.43 | 0.80 | 0.18 | 0.88 |

| | Ash | Protein | Lipid | Moisture |
|---|---|---|---|---|
| **Raw** | 11.92±2.79 | 4.91±1.56 | 0.16±0.04 | 77.64±8.02 |
| **Fried** | 8.70±2.69 | 28.79±4.60 | 12.71±3.42 | 34.89±7.89 |
| **Boiled** | 8.94±2.33 | 6.80±1.62 | 0.53±0.03 | 75.18±9.83 |
| **F value** | 2.83 | 38.45 | 43.28 | 83.22 |
| **p value** | 0.07 | 0.00 | 0.00 | 0.00 |

### 3. *Rastrelliger kanagurta*

| | Wet | Dry | F value | p value |
|---|---|---|---|---|
| **Potassium** | 0.29±0.12 | 7.15±4.92 | 13.11 | 0.00 |
| **Sodium** | 0.34±0.24 | 4.13±1.87 | 10.89 | 0.00 |
| **Calcium** | 0.22±0.07 | 0.14±0.07 | 0.01 | 0.94 |
| **Magnesium** | 0.16±0.05 | 0.54±0.11 | 5.53 | 0.03 |

| | Wet | Dry | F value | p value |
|---|---|---|---|---|
| **Ash** | 6.30±3.57 | 9.92±4.29 | 0.67 | 0.42 |
| **Protein** | 16.84±10.73 | 13.18±9.65 | 3.67 | 0.06 |
| **Lipid** | 6.86±2.08 | 3.69±1.99 | 12.36 | 0.00 |
| **Moisture** | 56±12.81 | 63.03±17.05 | 2.42 | 0.13 |

| | Potassium | Sodium | Calcium | Magnesium |
|---|---|---|---|---|
| **Raw** | 8.41±2.91 | 3.77±1.88 | 0.20±0.07 | 0.49±0.15 |
| **Fried** | 3.86±1.86 | 2.55±0.84 | 0.15±0.06 | 0.45±0.16 |
| **Boiled** | 2.55±0.52 | 2.32±0.52 | 0.16±0.05 | 0.31±0.14 |
| **F value** | 2.62 | 0.63 | 0.62 | 1.49 |
| **p value** | 0.11 | 0.54 | 0.55 | 0.26 |

| | Ash | Protein | Lipid | Moisture |
|---|---|---|---|---|
| **Raw** | 10.23±3.51 | 6.36±1.11 | 0.48±0.05 | 73.26±4.22 |
| **Fried** | 6.75±1.89 | 31.51± | 13.69±3.25 | 35.32±6.58 |
| **Boiled** | 7.44±2.04 | 7.14±1.86 | 1.70±0.09 | 69.84±6.64 |
| **F value** | 3.12 | 58.43 | 42.17 | 75.53 |
| **p value** | 0.05 | 0.00 | 0.00 | 0.00 |

### 4. *Siganus canaliculatus*

| | Wet | Dry | F value | p value |
|---|---|---|---|---|
| **Potassium** | 0.32±0.11 | 9.78±3.40 | 24.96 | 0.00 |
| **Sodium** | 0.54±0.28 | 4.87±1.97 | 18.01 | 0.00 |
| **Calcium** | 0.18±0.06 | 0.11±0.07 | 0.32 | 0.58 |
| **Magnesium** | 0.17±0.05 | 0.45±0.12 | 2.38 | 0.14 |

| | Wet | Dry | F value | p value |
|---|---|---|---|---|
| **Ash** | 8.86±3.26 | 11.09±3.54 | 0.493 | 0.49 |
| **Protein** | 18.61±9.69 | 10.77±4.95 | 20.267 | 0.00 |
| **Lipid** | 5.77±2.61 | 2.61±1.34 | 12.851 | 0.00 |
| **Moisture** | 57.53±16.15 | 67.14±14.90 | 19.314 | 0.00 |

| | Potassium | Sodium | Calcium | Magnesium |
|---|---|---|---|---|
| **Raw** | 7.54±2.77 | 3.10±1.06 | 0.09±0.02 | 0.34±0.14 |
| **Fried** | 4.23±1.06 | 2.44±0.96 | 0.16±0.04 | 0.33±0.11 |
| **Boiled** | 4.86±1.41 | 2.90±1.01 | 0.16±0.05 | 0.27±0.09 |
| **F value** | 0.75 | 0.12 | 2.64 | 0.34 |
| **p value** | 0.49 | 0.89 | 0.10 | 0.72 |

| | Ash | Protein | Lipid | Moisture |
|---|---|---|---|---|
| **Raw** | 12.00±2.43 | 4.96±0.70 | 0.22±0.04 | 77.13±5.01 |
| **Fried** | 8.53±2.39 | 32.25±5.42 | 11.87±3.58 | 36.14±8.25 |
| **Boiled** | 9.41±2.65 | 6.87±1.84 | 0.49±0.05 | 73.73±6.75 |
| **F value** | 3.53 | 46.14 | 37.57 | 69.06 |
| **p value** | 0.04 | 0.00 | 0.00 | 0.00 |