

**EFFECTS OF PROCESSING ON NUTRIENT COMPOSITION, SHELF-LIFE  
AND PROTEIN-BIOAVAILABILITY OF ANCHOVIES FISH FROM  
INDIAN OCEAN (*Stolephorus heterolobus*)**

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## ABSTRACT

This study assessed changes in chemical components of the sun dried and hot smoked anchovies processed by traditional and improved methods. Except for the protein-bioavailability analysis which took place once during the fourth week, the samples of fish were assessed weekly. Nutrient composition, shelf-life and protein-bioavailability of processed anchovies were assessed using changes in proximate composition and specific minerals, biochemical indices and protein-digestibility, respectively. The pH levels decreased and TVB-N increased with the number of weeks. These situations implied that spoilage of anchovies occurred gradually. Changes in moisture content, crude protein and calcium indicated significant differences ( $p \leq 0.05$ ), in terms of processing methods (hot-smoking against sun-drying). The packaging aspects were not significantly different for all parameters ( $p > 0.05$ ). In categories of processing methods (traditional versus improved methods), the mean values of crude protein, crude fat and zinc were significantly different ( $p \leq 0.05$ ). The mean protein-digestibility values (73.8%) for each pair of processing, packaging and categories of processing aspects were not significantly different from each other ( $p > 0.05$ ). The implication is that the availability of protein by the healthy human body depends on increase in nutrient-density. It was concluded that the protein-density of fish products processed by improved methods was higher than that of traditional methods. Based on moisture contents of anchovies processed by improved methods, their shelf-life could be extended for more than a month. It was recommended that moisture content less than 10% should be maintained to reduce

microbial contamination. The use of hot smoking should be encouraged in order to dry the fish throughout the year.

**DECLARATION**

I, HASHIM ALLY TENGENI, do hereby declare to the Senate of Sokoine University of Agriculture that, this dissertation is my own original work and that it has not been nor is concurrently being submitted for a higher degree and to any other institution.

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## **DEDICATION**

This dissertation is dedicated to my late parents, Ally Tengen and Zena Omary, my wife, Z. R. Maumba and my daughters, Asia and Rahma.

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**LIST OF ABBREVIATIONS AND SYMBOLS**

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CEO	Chief Executive Officer
CFS	Committee on World Food Security
DCC	Dar – Es – Salaam City Council
FAO	Food and Agriculture Organization
FETA	Fisheries Education and Training Agency
FFA	Free Fatty Acid
ICF	Inner City Fund
IFIC	International Food Information Council Foundation
LCPUFA	Long Chain Poly Unsaturated Fatty Acid
LSD	Least Significant Difference
MLFD	Ministry of Livestock and Fisheries Development
NBS	National Bureau of Statistics
PUFA	Poly-unsaturated Fatty Acids
SFOL	Sea Food Open Learning
SPSS	Statistical Package for Social Science
SUA	Sokoine University of Agriculture
TVB	Total Volatile Bases
TVB-N	Total Volatile Basic-Nitrogen
WFC	World Fish Centre

## CHAPTER ONE

### 1.0 INTRODUCTION

#### 1.1 Background Information

Anchovies are a family (Engraulidae) of small, common salt-water forage fish. Anchovies are known as “*Dagaa mcheli*” in Swahili and are caught by fishing gears such as purse seines, ring nets, stake nets and dip-nets, having maximum length of 8cm (Bianchi, 1985). The nutritional importance of fish includes: decreasing the risk of cardiovascular disease, cancers and asthma (Marin *et al.*, 2010), facilitating infant neurodevelopment, human fertility, strong bone and teeth formation (Novak, 2012). Besides, fish is an excellent source of protein, minerals and vitamins (IFIC, 2009). In sub-Saharan Africa, fish provides 22% of the protein intake (WFC, 2005), while in the poorest countries it can exceed 50%. In Tanzania, the contribution of fish to the total animal protein intake is about 30% (Nazir *et al.*, 2010).

Fish is an extremely perishable food that could become inedible after twelve hours of fishing at tropical temperatures. Spoilage begins as soon as the fish dies, and processing should therefore be done quickly to prevent the growth of spoilage bacteria (Berkel *et al.*, 2005). Other factors of fish spoilage are enzymatic actions and fat oxidation (SFOL, 2010). The fish spoilage may cause a net reduction in the amounts of nutrients potentially available to the consumer either by direct physical or nutritional loss (Daramola *et al.*, 2007). Several processing methods (e.g. freezing and drying) are carried out to extend the shelf-life of fish and fishery products.

Traditional fish processing methods in Tanzania include smoking, sun drying and deep frying (MLFD, 2012).

These methods can be used in improved forms such as sun-drying by using a solar tent dryer and hot-smoking by using altona kiln. Despite the fact that fish processing adds value and extends shelf-life, still deterioration and spoilage can occur in processed fish along the fish value chain if not properly monitored. According to Koroma (2012) an average of 10% of the total fish catch in Zanzibar is lost annually in processing, preservation and storage.

In Tanzania, the shelf-life of the fish faces many challenges such as un-hygienic fishing, handling, processing and poor storage (Diei-Ouadi and Mgawe, 2011). Improved processing, handling and storage of the fish product contribute to an increase in its shelf-life. Packaging can play part in enclosing or protecting fish products for distribution, storage, sale and use, so as to prolong the shelf-life (Soroka, 2002).

Fish is a very perishable commodity and hence susceptible to high post-harvest losses. Both physical and quality losses are high in fisheries sector and these translate into losses in nutritional contribution of fish to the total diet and health of population (Kabahenda *et al.*, 2009). If the nutrient composition, bioavailability and shelf-life of the product are negatively affected by their respective factors, then the consumer may face problems such as mal-absorption and under nutrition. In other words,

contribution of the fish product towards the diet of consumer will be low (Ames, 1992).

### **1.2 Problem Statement**

Although various initiatives to improve nutritional status of people have been taken globally the number of undernourished people are still on the rise. It increased from 843 million in 2003-05 to 1,020 million in 2009. Under nutrition occurs when people do not eat (or absorb) enough nutrients to cover their needs for energy and growth, or to maintain a healthy immune system (Burgess and Danga, 2012). It is a direct result of insufficient food intake or repeated infectious diseases or combinations of both (Kawarazuka, 2010).

This situation can be prevented by providing individuals with balanced diet containing right amount of proteins, carbohydrates, lipids, vitamins and minerals. In Tanzania, a survey done in 2005 and reported by NBS and ICF (2011) indicated that 38% of the children under 5 were moderately stunted and 13% were severely stunted, where as the 2009/10 survey disclosed that 42.3% of children under 5 were moderately stunted and 16.6% were severely stunted.

### **1.3 Justification of the Study**

Anchovies are one of the good sources of nutrients like protein, fat, vitamins and minerals. Although they are good sources of nutrients, poor processing methods, as well as the presence of anti-nutritional factors may render these nutrients

unavailable. Therefore to ensure availability of these nutrients, proper processing methods are a pre-requisite. It is therefore very important to assess effects of processing methods on nutrient composition, bioavailability and shelf-life of anchovies.

#### **1.4 Objectives of the Study:**

##### **1.4.1 Overall objective:**

To assess the effects of sun-drying and hot smoking methods on nutrient composition, protein-bioavailability and shelf-life of anchovies.

##### **1.4.2 Specific objectives:**

- 1) To evaluate nutrient composition of processed anchovies from traditional (sun-drying) and improved processing methods (sun-drying and hot-smoking) throughout the shelf-life.
- 2) To determine protein bioavailability of processed anchovies from traditional and improved processing methods throughout the shelf-life.
- 3) To determine shelf-life of anchovies processed by traditional and improved methods.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Descriptions of Anchovies (*Stolephorus heterolobus*)

Anchovies are a family (Engraulidae) of small, common salt-water forage fish. There are 144 species in 17 genera, found in the Atlantic, Indian, and Pacific Oceans. Anchovies are usually classified as oily fish, small and green with blue reflections due to a silver longitudinal stripe that runs from the base of the caudal fin (Wikipedia, 2014). Anchovies are known as “*Dagaa mcheli*” in Swahili and caught by fishing gears such as purse seines, ring nets, stake nets and dipnets, having maximum length of 8cm (Bianchi, 1985). “*Dagaa*” is the collective Swahili name in Tanzania for various types of sardine-like fish eaten in a dried form by poor and middle-income groups throughout eastern and southern Africa (Gibbon, 1997).

#### 2.2 Quality Value of the Anchovies

##### 2.2.1 Nutritional quality of fresh anchovies

Fish provides a good source of high quality protein and contains many vitamins and minerals. Fish may be classified as either white, oily or shellfish. White fish, such as haddock and seer, contain very little fat (usually less than 1%) whereas oily fish, such as sardines and anchovies, contain between 10 - 25%. The latter, as a result of its high fat content, contain a range of fat-soluble vitamins (A, D, E and K) and essential fatty acids, all of which are vital for the healthy functioning of the body (Fellows and Hampton, 1992). A live fish has natural defense mechanisms that help

to prevent spoilage. However, once a fish dies, its defense mechanisms stops and enzymatic, oxidative and microbiological spoilage begins to cause quality deterioration (Diei-Ouadi and Mgawe, 2011). If fish is not sold fresh, preservation methods such as freezing, smoking, sun drying and heat treatment should be applied in order to extend shelf-life (Bykowski and Dutkiewicz, 1996).

### **2.2.2 Nutritional quality of dried/processed anchovies**

The dried sardines (*Dagaa*) are rich in protein, fat, Iron, Zinc and Calcium in because these fish are eaten whole (Kabahenda *et al*, 2011). Nutritional losses can occur in fresh fish and some loss of nutrients is inevitable in all forms of food processing. Fish processing is no exception and in traditional methods, the losses may be serious (Kumolu-Johnson and Ndimele, 2011). Due to the high levels of long chain polyunsaturated fatty acids (LCPUFAs), fish products are susceptible to oxidation. Oxidation of lipids is associated with a decrease in triacylglycerols and phospholipids and an increase in free fatty acids and often results in a product with off flavors (rancid) which may not be appealing to many consumers (Kabahenda *et al*, 2009).

The bacteria which cause the spoilage themselves eat the protein which is intended to be the main human food. This is not generally a major factor, as bacterial action generates nitrogenous substances with objectionable smells. The fish will become highly unattractive because there is too much nutritional damage. It could be more significant in freshwater fish, when less violent ammoniacal odours are produced than in marine fish, where tri-methyl-amine and its breakdown products rapidly

impart strong odours (Kumolu-Johnson and Ndimele, 2011). In improved fish processing methods, proper packaging is incorporated so as to protect the processed fish from an attack or damage by microbes and enzymatic activities (Abolagba and Akise, 2011).

## **2.3 Chemical Composition, Protein-bioavailability and Shelf-life of processed Anchovies**

### **2.3.1 Chemical composition of processed anchovies**

Proximate composition of fish varies with Species, body size, season, environmental factors and nutritional status (Sankar and Ramachandran, 2001). Huss (1995) reported that the chemical composition of different fish species shows variation, depending on season, migratory behavior, sexual maturation and feeding cycles.

According to Daramola (2007), the crude protein forms the largest quantity of the dry matter in the fish. The initial crude protein can be reduced to more volatile products such as total volatile bases (TVB), hydrogen sulphide and ammonia (Eyo, 2001). Fish at 10 - 15% moisture content has a shelf-life of 3 - 9 months when stored properly (Jallow, 1995). The lipid in fish tissue contains poly-unsaturated fatty acids (PUFA) which can be oxidized to products such as peroxides, aldehydes, ketones and the free fatty acids, consequently reducing the lipid content (Horner, 1992). Fish oil has been found to be more liable to spoilage than other oils due to their greater number of unsaturated fatty acids (Eyo, 1993).



### **2.3.2 Protein-bioavailability of processed anchovies**

Digestibility refers to the quantitative aspect of the digestive process. It's a measure of the nutritional usefulness of food or availability of the nutrient component to the fed species (Sultana *et.al*, 2010).

Ramos *et.al* (2012) reported that in vitro protein digestibility in raw anchovy was  $79.2 \pm 1.01\%$ . El and Kavas (1996) reported that protein digestibility is reduced as a result of complex chemical (cross-linking) reactions, such as protein interactions or protein-fat interactions when food was boiled at high temperatures.

### **2.3.3 Shelf-life of processed anchovies**

To store food properly, a person needs to know not only how to store, but also how long they will be safe and of high quality (Laanen, 2000). Eyo (1993) stated that pH is an indicator of the extent of microbial spoilage in fish and that some proteolytic microbes produce acid after decomposition of carbohydrate, thereby increasing the acid level of the medium. The ratio of Volatile Basic Nitrogen to the Total Nitrogen has been recommended as a useful index of quality in fish (Huss, 1988). Pearson (1982) recommended that the limit of acceptability of fish is 20 - 30mg N per 100g while Kirk and Sawyer (1991) suggested a value of 30 - 40mg N per 100g as the upper limit, and Connel (1995) reported the limit of acceptability to be 30mg N per 100g.

The Peroxide value is a primary indicator of oxidation of fat (rancidity). According to Daramola *et.al* (2007) the peroxide values corresponding to incipient spoilage are

usually in the order of 20 - 40 milliequivalents of oxygen per Kg of sample (ml per Kg). However, Connell (1995) reported that when peroxide value is above 10-20 ml per Kg, fish develop rancid taste and smell. Free Fatty Acid (FFA) is a tertiary product of rancidity. It is a measure of hydrolytic rancidity-the extent of lipid hydrolysis by lipase action. In most fish oils, rancidity is noticeable when the FFA (calculated as oleic acid) is in between 0.5 - 1.5% (Eyo, 1993).

#### **2.4 Fish Drying Methods**

Fish salting and drying decrease the water activity and consist of transporting salt into food structures and is governed by various physical and chemical factors such as diffusion, osmosis and a series of complicated chemical and biochemical processes. The common species which are subjected to dried fish production in India are oil sardines, lesser sardines, tuna, silver bellies, mullets, mackerels and ribbon fishes (Logesh *et al*, 2012). Traditional fish processing methods, in East Africa, include: hot smoking involving stacking fish on wire meshes and then smoking them over open fire for one to three days; sun drying which involves exposing the fish to heat from the sun by placing the products either directly on the ground, on mats placed on the ground or on racks; deep frying using oils which are highly susceptible to oxidative rancidity (Kabahenda *et al.*, 2009).

The major purpose of fish processing and preservation is to prolong the shelf-life through prevention and or inhibition of spoilage factors such as microorganisms, enzymes and air (oxygen). In accordance with Leroi *et al.* (2006), the microbial safety and stability of most food, are based on an application of preservative factors

called hurdles. Examples of preservative factors in marine products are salt, smoke, acids, temperature, fermentative microorganisms and more recently redox potential (vacuum-packed products). Presence of each preservative factor implies putting microorganisms in a hostile environment, which inhibits their growth or causes their death (Leistner, 2000).

#### **2.4.1 Sun drying**

Sun drying of fishes is a simple and the oldest known method of fish preservation where fish are dried under the sun. The drying method is considered as the least expensive method of fish preservation (Logesh *et al*, 2012). The storage life of low quality sun-dried “*dagaa*” is 1.5 to 2 months, whereas salted dried fish can be stored for 2 to 3 months at ambient temperature (Bashemererwa, 1992). The dried-salted fish, having salt content of 10–15%, successfully inhibits fish spoilage, but may be a limiting factor to consumer acceptance. Some vitamins are sensitive to heat, sunlight and water, while other nutrients such as protein, fat, iron and calcium are stable, even after processing and cooking (Kawarazuka, 2010). According to Roos *et al.* (2003), almost all vitamin A in small fish is destroyed after sun-drying. The combination of foods eaten together with fish in a meal is an important factor to determine bioavailability and total dietary intake. For instance, when small fish are cooked with some oil, chilli, lemon, tamarind and vegetables (Roos, 2001), they contribute to vitamin C and additional vitamin A from the other foods eaten with the fish and in turn vitamin C from vegetables and spices enhance bioavailability of iron (Kawarazuka, 2010).

### **2.4.2 Hot smoking**

The preservative effect of the smoking process is due to drying and the deposition in the fish flesh of the natural chemicals of the wood smoke. Smoke from the burning wood contains a number of compounds which inhibit bacterial growth. Heat from the fire causes drying, and if the temperature is high enough, the flesh becomes cooked. Both of these factors prevent bacterial growth and enzyme activity which may cause spoilage (Fellows and Hampton, 1992). When stored at ambient temperature, the smoked fish can be consumed within four weeks (Daramola *et al.*, 2007). The “*dagaa*” sun-drying is affected by heavy and prolonged rains in Tanzania, so the alternative method for preservation of “*dagaa*” is the hot smoking which leads to supply of the product all year round (Bille and Shemkai, 2012). Heating protein foods can cause loss of nutritional value through amino acids destruction, protein denaturation and maillard reaction. The digestibility of most amino acids increases due to an unfolding of protein under the mild heating (60 °C to 100 °C), which favours the formation of an enzyme-substrate complex and allows a more rapid cleavage of peptide linkages (Jangam *et al.*, 2010)

### **2.5 The Effects of Processing on the Chemical Composition of Fish**

Smoking is meant to preserve fish by reducing moisture content; however, the traditional smoking process is associated with unavoidable and large losses of nutrients. Before smoking, fish products are placed on racks in the kiln and allowed to drip dry for several hours, which results in some nutrients being lost in the exudate and the process also fosters microbial growth that induces proteolysis. During the smoking process, fat and more water drips from the fish resulting in physical loss of

lipids (including essential fatty acids) and micronutrients. Hot smoking at high temperatures degrades protein, reduces functionality of essential amino acids and may lead to loss of important nutrients such as antioxidants (Kabahenda *et al.*, 2009).

Procedures in which the fish is heated to fairly high temperatures, such as smoking, can result in damage to the nutritional value of fish protein, with losses in availability of lysine and other essential amino acids (Kumolu-Johnson and Ndimele, 2011). On dry weight basis, protein is the most dominant biochemical constituent of the fish and is not affected by processes of smoking and sun-drying whereas lipids, ash, fibre and carbohydrate are impacted by both (Akintola *et al.*, 2013).

Nutrients are the substances, chemical elements and compounds that food contains. They make the body grow; maintain our bodies in good repair. They provide energy and keep the body healthy (CFS, 2012). The quality of food does not only depend on the nutrient content but also on their bio-availability. Bioavailability is defined as a proportion of nutrient in food that can be utilized for normal body function (Promchan and Shiowatana, 2005). Nutrient bioavailability can be affected by various factors including the type and composition of food (e.g. anti-nutritional factors, components interactions), different processing/cooking procedures and gastrointestinal conditions (Intawongse and Dean, 2006). Assessment of protein bioavailability is taken as the means of protein quality determination; and sometimes accompanied by determination of amino acids composition and digestibility of protein (Hoffman and Falvo, 2004).

## CHAPTER THREE

### 3.0. MATERIALS AND METHODS

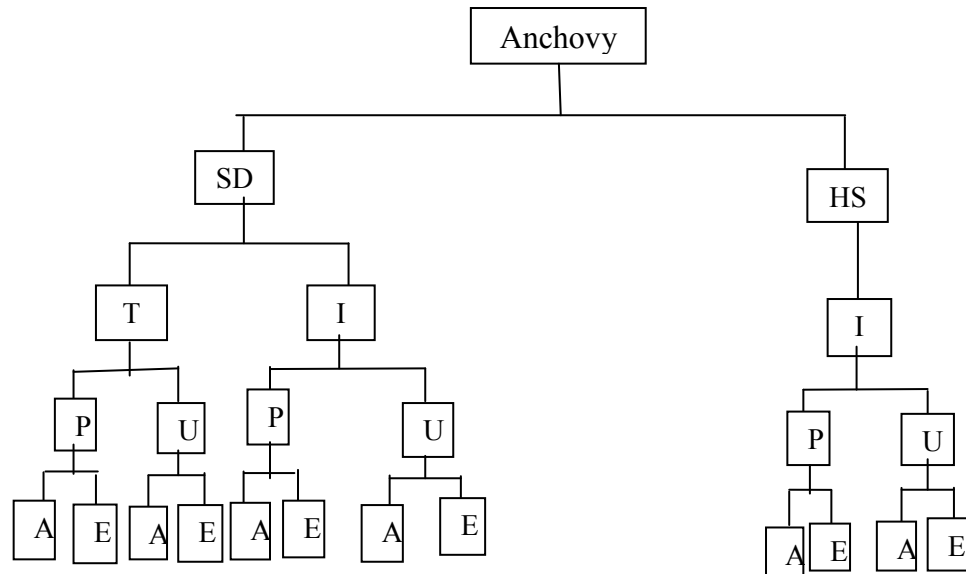
#### 3.1. Description of the Study Location

The study was conducted in Ilala district, Dar-es-Salaam and Unguja/Urban district, Zanzibar Urban/West. Ilala is among the three districts of Dar-es-Salaam city, namely: Kinondoni, Temeke and Ilala. Dar-es-Salaam is in the eastern part of Tanzania, and located between latitudes  $6.36^{\circ}$  and  $7.0^{\circ}$  to the south of Equator, and longitudes  $39.0^{\circ}$  and  $33.33^{\circ}$  to the east of Greenwich. It is bounded by the Indian Ocean on the east and by the Coast Region on the other sides. The major economic activities in Dar es Salaam include: internal trade; manufacturing; tourism; transport and communication; urban agriculture; forestry and fishing; mining and quarry; utility services; construction; finance and insurance; public administration and education (DCC, 2004). Unguja/Urban is one of the two districts of Zanzibar Urban/West region, namely: Unguja/Urban and Unguja/West. The region is located in the eastern part of Unguja Island (Zanzibar). The coordinates, in which the region is situated, are  $6^{\circ}10'S$   $39^{\circ}14'E$  (Wikipedia, 2012).

#### 3.2. Study Design

The study consisted of factorial experimental designs. The study design was applied in laboratory analyses for the treatments of samples. In this case, three factors were examined, namely: the fish processing methods – 2 levels (sun drying and hot smoking); and processing categories – two levels (traditional and improved

methods); and packaging aspects (packaged and un-packaged). Three variables were assessed, from each of the two processing methods, including chemical composition, protein bioavailability and shelf-life. Figure 1 below summarizes the experimental design for both hot smoked and sun-dried anchovies during storage time.



**Figure 1: Schematic presentation of the research work**

Key:

SD = Sun drying    T = Traditional method, A = Analysis    E = Enzyme Digestion

HS = Hot smoking    I = Improved method

P = Packaged    U = Un-packaged,

A total weight of 60 kg of anchovies was used in this study

### **3.3. Sample Collection**

#### **3.3.1. Sampling procedure**

Samples of sun-dried anchovies were bought from artisanal fish processors at Ferry and Kizingo fish markets in Dar-es-Salaam and Unguja Urban/West regions, respectively.

The sample of fresh anchovies was bought from fishermen at Ferry fish market and put on ice flakes in thermo cooling boxes immediately after collection in order to retard spoilage during transportation. The anchovies were transported to Fisheries Education and Training Agency (FETA) – Bagamoyo Campus for further processing. At FETA – Bagamoyo Campus, the fish were stored in a deep freezer waiting for processing the following day.

#### **3.3.2 Processing of anchovies**

The sample of frozen fish was subdivided into two equal parts. One part was subjected to hot smoking using Altona kiln, whereas another part was sun-dried by using solar tent drier. Processing of anchovies was carried out on the processing site after delivery. Only, clean, sound fish, which were suitable for human consumption, were used for processing. Both sun-dried and hot smoked anchovies were divided into two batches each. In each case, one portion of processed anchovies was hygienically packaged in a clean transparent polyethylene bags and covered with paper boxes after cooling, while another portion was left open in the clean paper boxes. Processed fish samples were stored at ambient temperature (25 °C – 30 °C).



The improved post harvest drying methods for anchovies such as hot smoking by using the Altona kiln and sun drying using the solar tent drier were carried out at FETA – Mbegani Campus.

All those samples were transported to Nyegezi – Mwanza and SUA – Morogoro for chemical analyses. The open shelf and packaged anchovies were placed in the covered and clean paper boxes. Sun-dried anchovies from artisanal processors were termed as fish processed using traditional methods, whereas, the sun dried anchovies (Solar tent dryer) and hot smoked anchovies were categorized as fish processed by improved methods.

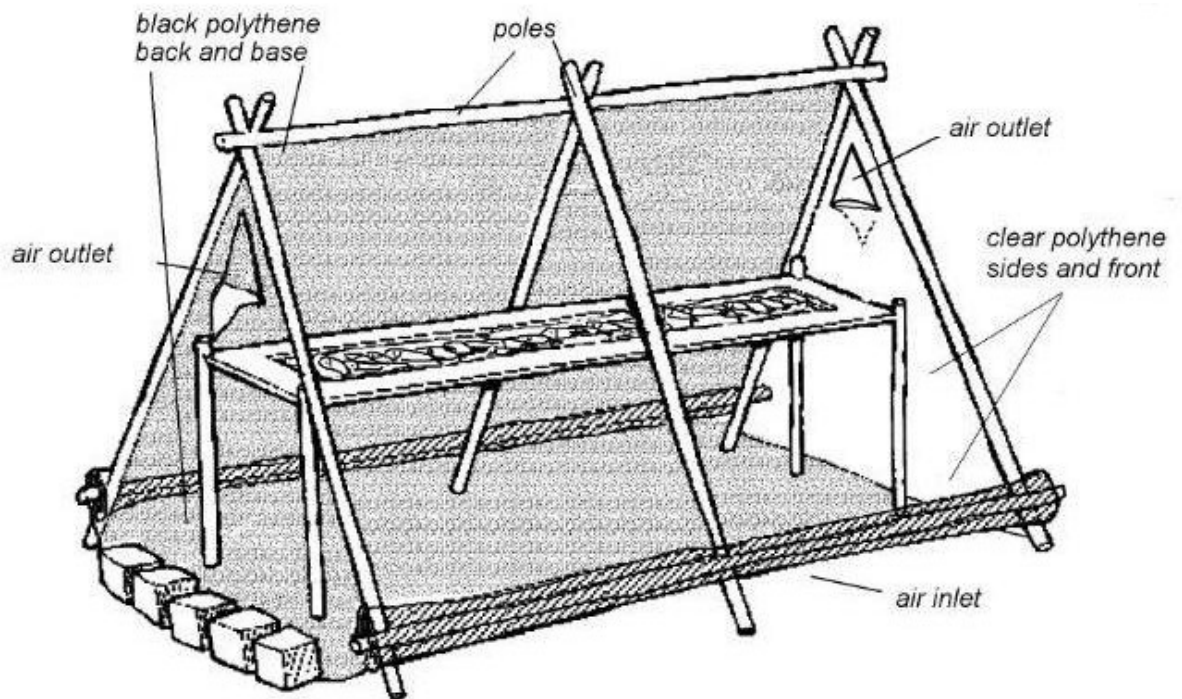
### **3.3.2.1 Simulation of current traditional processing methods for anchovies in controlled manner**

The simulation of the fish drying methods was done by adopting all steps found in traditional methods except handling and drying steps. The handling step in improved methods involved the uses of ice and deep freezer. The drying step was performed in improved manner as described in sun drying and hot smoking for the fish by Berkel *et al.*, (2005) and Mhongole and Mhina (2012), respectively.

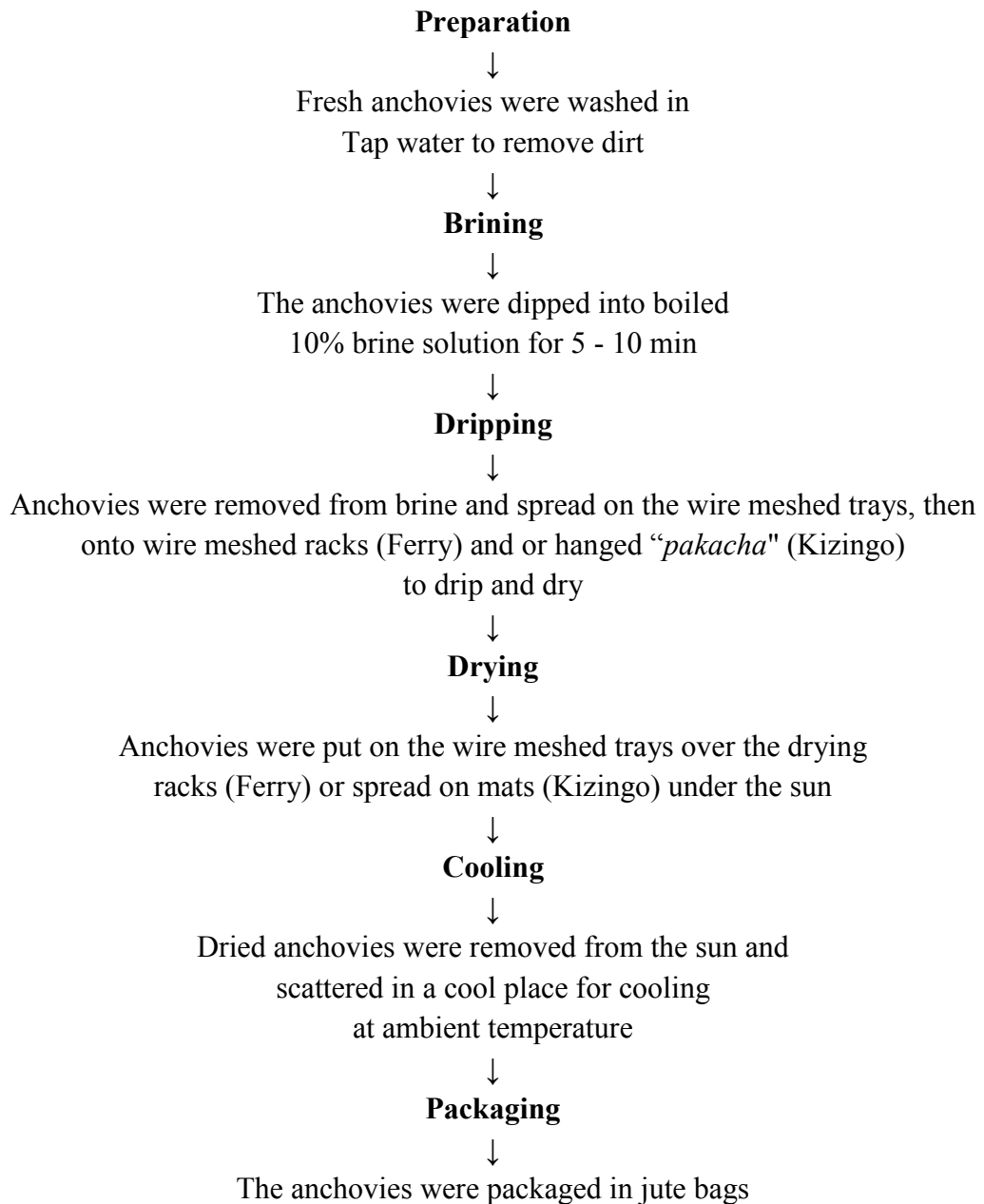
#### **3.3.2.1.1 The sun-drying of anchovies by using the solar tent dryer**

The fresh fish from the freezer were thawed and washed. The brine solution (10% w/w concentration) was prepared by dissolving 300g of fine salt into 2.7 liters of water. The brine was boiled in the pan. The fish were placed in the stainless steel strainer and then into the boiling brine for 30 seconds. The brined products were

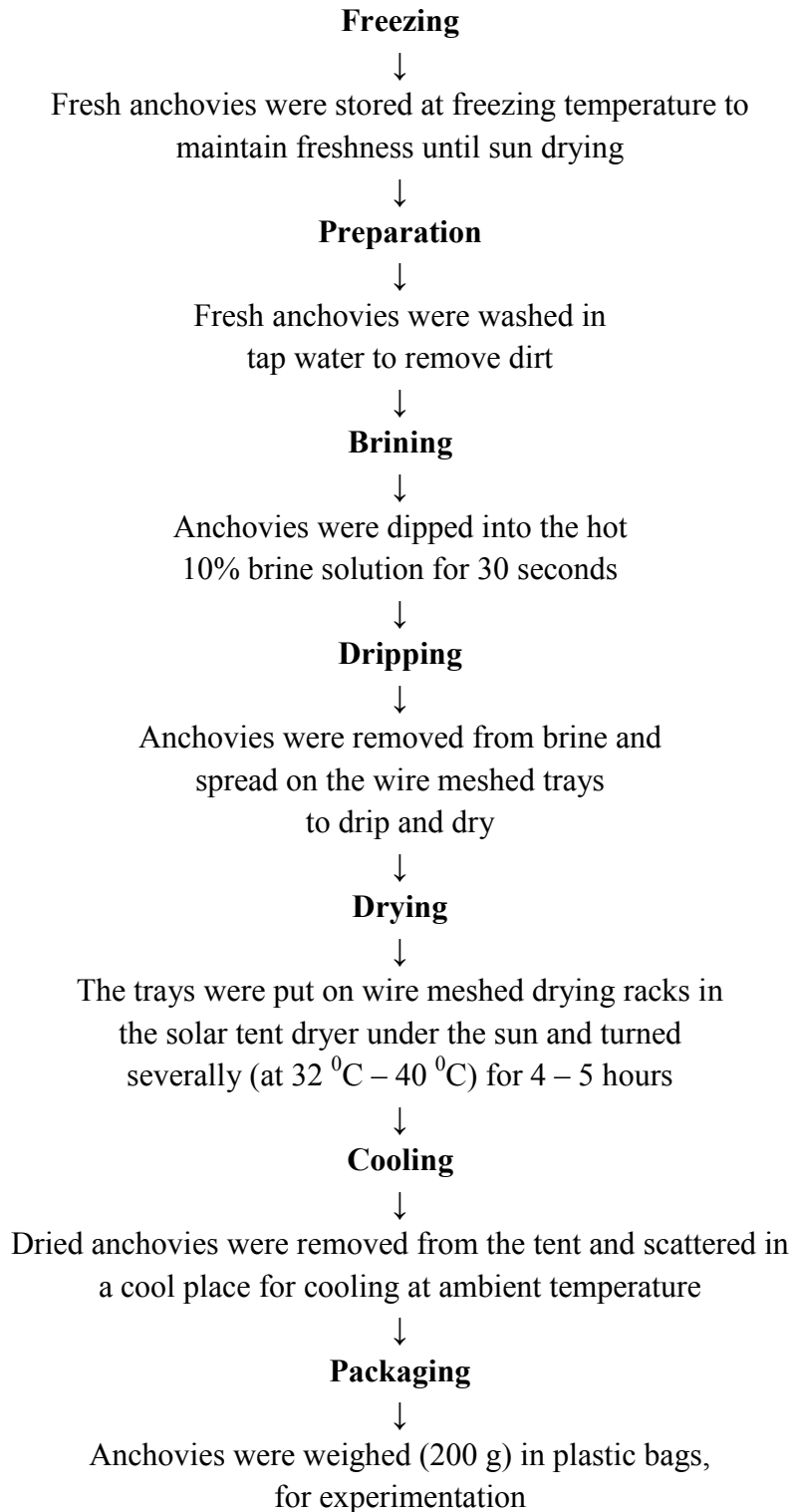
placed and spread on the drying rack inside the solar tent dryer as shown in figure 2. The temperature in the solar tent dryer ranged between 32°C – 40°C. The drying of the fish products took 4 -5 hours. The products were cooled on the stainless steel table for an hour, ready for packaging. Packaging was done manually and hygienically in the transparent polyethylene paper bags, inside the covered paper box. The un-packaged portion of the product was placed in the open paper box (Berkel *et al.*, 2005).



**Figure 2: Sketch of the solar tent dryer (Source: Berkel *et al.*, 2005)**



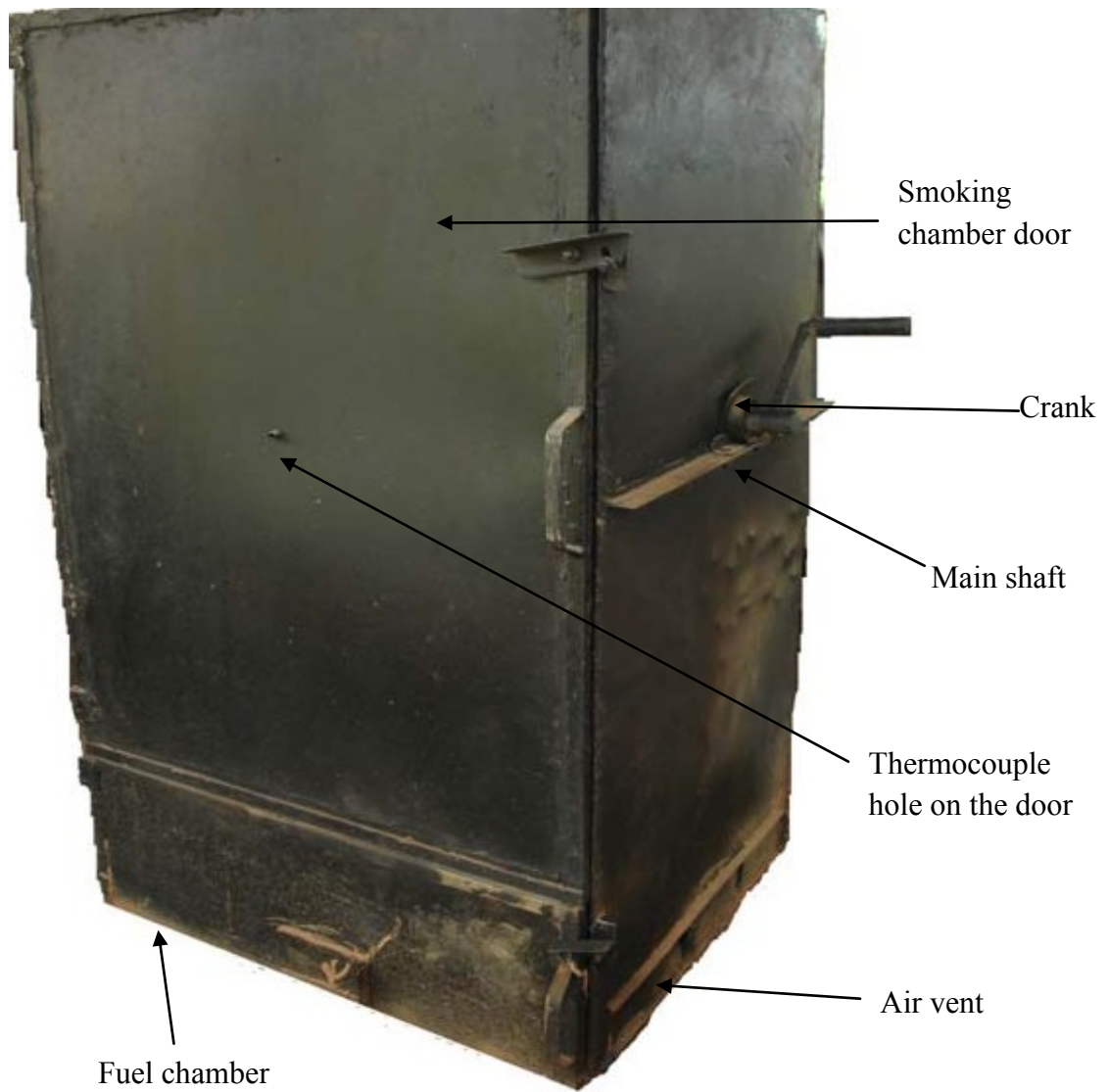
**Figure 3: The flow diagram for traditional processing methods of anchovies - Open sun-drying**



**Figure 4: The flow diagram for improved processing methods of anchovies - Sun-drying using the solar tent dryer**

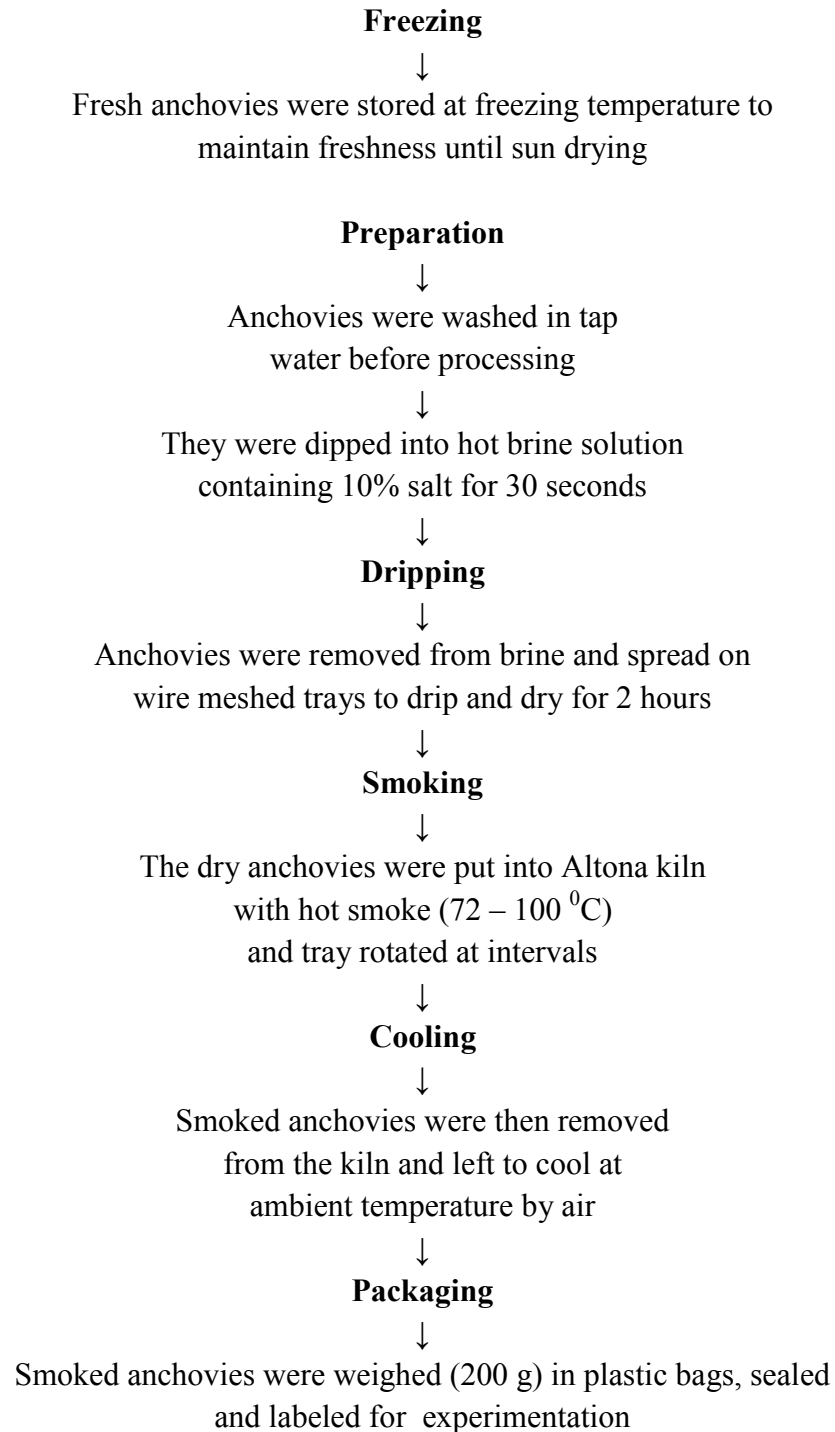
**3.3.2.1.2 The hot smoking of anchovies by using the Altona kiln**

The fresh fish from the freezer were thawed, by sealing them in a plastic bag and immersing them in cold water, and washed. The brine (10% w/w concentration) was prepared as done for sun-drying. The brine was boiled in the pan. The fish were placed in the stainless steel strainer and then into the boiling brine for 30 seconds. The brined products were placed and spread on the raised wire mesh racks in order to drain some water for 2 hours. After draining the fish, the racks were put into Altona kiln for hot smoking.



**Figure 5: A diagram of Altona kiln oven (Source: Oyerinde *et al.*, 2013)**

Fire woods from hardwood were used as the source of heat and smoke. Inside the kiln, the fish were smoked for an hour. Afterwards, the fish products were cooked in the kiln at temperature between  $70^{\circ}\text{C}$  –  $100^{\circ}\text{C}$  for an hour. The smoked products were cooled for an hour, on the stainless steel table, ready for packaging. The packaging took place in the same way as in the case of sun drying using the solar tent dryer (Mhongole and Mhina, 2012).



**Figure 6: The flow diagram for improved processing methods of anchovies – Hot smoking using the Altona kiln**



### **3.3.3. Sample preparation for chemical analysis**

Processed anchovies samples were stored in clean non transparent polyethylene bags inside the covered paper boxes and open paper boxes. They were then ground right before every assessment of the nutrient composition, protein-bioavailability and shelf-life. Except for the samples meant for protein-bioavailability analysis which were analyzed in the last (fourth) week only, the rest were analyzed on weekly basis for four consecutive weeks.

## **3.4. Analysis of Samples**

### **3.4.1. The nutrient composition, protein bioavailability and shelf storage analyses**

The fish samples were analyzed at Nyegezi Fish Quality Control Laboratory (NFQCL) Mwanza and Sokoine University of Agriculture (Animal Science Department) laboratory, Morogoro. The nutrient composition and shelf-life analyses were carried out at NFQCL – Mwanza. The analysis for protein-bioavailability was done at SUA (Animal Science Department) Laboratory. The analyses were performed as shown in subsections 3.4.2, 3.4.3 and 3.4.4.

### **3.4.2. Evaluation of nutrient composition of processed anchovies by traditional and improved methods**

All experiments on determination of proximate composition and mineral contents (calcium, iron and zinc) were performed in duplicate. The proximate composition such as moisture content, crude fat and crude protein of dried anchovies were

determined using the AOAC methods 950.46, 991.36 and 928.08 (AOAC, 1995), respectively. Minerals calcium, iron and zinc contents for processed sardines were determined quantitatively using the equipment known as VARIAN AA 280FS, according to Varian (1988).

#### **3.4.3. Determination of protein-bioavailability of processed anchovies**

Protein-digestibility was determined as in vitro protein-digestibility using pepsin enzyme according to AOAC Official Method 971.09 (Latimer, 2012).

#### **3.4.4. Determination of shelf-life of anchovies processed by traditional and improved methods**

Chemical changes in processed fish like pH and total volatile Basic-nitrogen (TVB-N) were assessed for four weeks, consecutively. The changes in pH were determined using HANNA pH 211 (Microprocessor pH meter), while the TVB-N was determined using the methods described by Pearson (1970). In each analysis, an average of duplicate trials or values was recorded.

#### **3.5. Data Analysis**

Data were subjected to ANOVA using the Statistical Package for Social Science (SPSS version 16). Comparisons of means were done using Least Significant Difference (LSD). A probability of  $< 0.05$  ( $p < 0.05$ ) was considered to be statistically significant.

## CHAPTER FOUR

### 4.0 RESULTS AND DISCUSSION

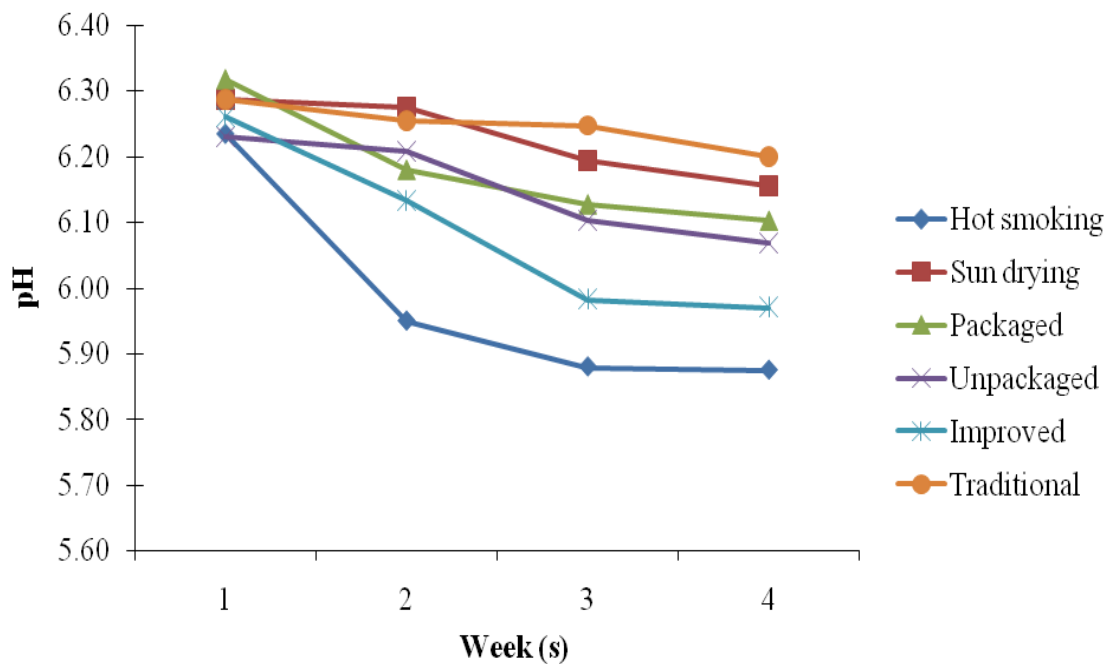
#### 4.1. Analyses of Shelf-life of Processed Anchovies

The results of pH and TVB-N analyses of the dried anchovies are presented in figures 7 and 8. The pH of the fish samples decreased weekly, towards acidic region.

Total volatile basic - Nitrogen (TVB-N) indicated gradual increase during storage.

##### 4.1.1. Changes in pH levels of the dried anchovies during storage

The results (in Figure 7) show the decrease in pH as the number of weeks increase for all aspects of processing and preservation.



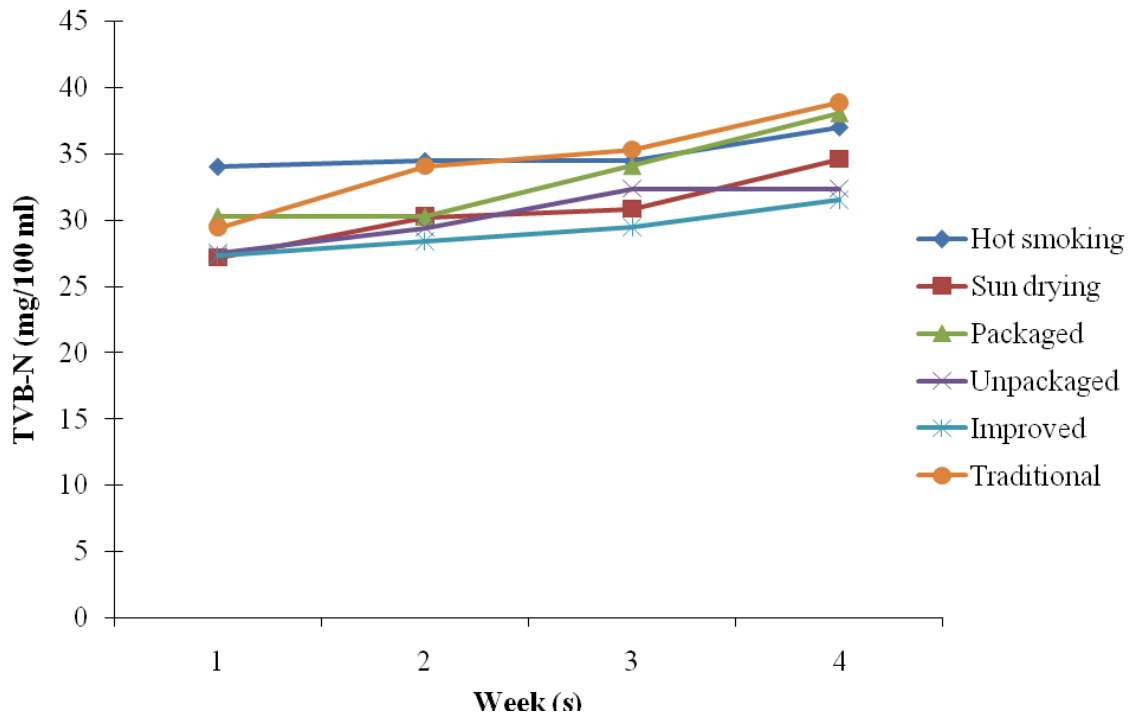
**Figure 7: Weekly changes in pH level of the dried fish during storage**

In the traditional category of processing methods pH decreased from 6.29 to 6.20; and for sun-drying methods the pH values decreased from 6.29 to 6.16. Packaged fish products pH values decreased from 6.32 to 6.10 and un-packaged fish products from 6.23 and 6.07. It was followed by improved methods having initial and final pH values of 6.26 and 5.97, respectively. The last aspect was hot-smoking method with initial and final pH values of 6.24 and 5.88.

This result indicated that the spoilage took place gradually for all samples. Eyo (1993) stated that pH is an indicator of the extent of spoilage in fish and that some proteolytic microbes produce acid after decomposition of carbohydrate, thereby increasing the acid level of the medium. The pH value is a reliable indicator of the degree of freshness or spoilage and the decrease in the pH level implies that carbohydrate of the fish is fermented to acids (Daramola *et al.*, 2007).

#### **4.1.2. Changes in TVB-N levels of the dried anchovies during storage**

The Total Volatile Basic Nitrogen (TVB-N) of the all samples of dried anchovies gradually increased during storage (Figure 8).



**Figure 8: Weekly Changes in TVB-N level of the dried fish during storage**

The TVB-N value for hot smoked anchovies changed from 34.02 mg N per 100g to 36.96 mg N per 100g. The sun-dried anchovies had initial and final TVB-N values of 27.16 mg N per 100g and 34.58 mg N per 100g respectively. The TVB-N value for packaged anchovies changed from 30.24 mg N per 100g (first week) to 38.01 mg N per 100g (fourth week). On the other hand, the initial and final TVB-N values for un-packaged anchovies were 27.51 mg N per 100g and 32.34 mg N per 100g, respectively. For the case of anchovies processed by improved methods, the TVB-N value changed from 27.30 mg N per 100g to 31.50 mg N per 100g. Meanwhile, the TVB-N value for anchovies processed by traditional methods increased from 29.4 mg N per 100g to 38.85 mg N per 100. On the bases of change in TVB-N, the most suitable products were those from improved category of processing methods.

The results in Figure 8 indicated the difference in the increase in TVB-N for each of the processing methods. This is because the initial TVB-N values of the processing and preservation aspects were different from each other. Otherwise, the traditional methods category and sun-drying methods indicated more rapid increase in TVB-N than other methods due to probably unhygienic handling and processing steps involved in traditional methods. In other words, traditional methods consisted of sun-drying methods while improved methods comprised sun-drying and hot-smoking methods.

#### **4.2. Nutrient Composition of Processed Anchovies**

The results of proximate and minerals assessment are shown in Tables 1 – 3 and Appendices 2, 11 and 20. Regarding the processing methods (hot smoking against sun-drying), changes in mean moisture content, crude protein and Calcium indicated a significant difference, at the 5% confidence level (Table 1 and Appendix 2) . Meanwhile, changes in average crude fat, Zinc and Iron were not significantly different at the 5% confidence level (Table 1 and Appendix 2). The packaging aspects (packaged against un-packaged anchovies) were not significantly different, at the 5% confidence level, for all parameters (Table 2 and Appendix 11).

For the case of categories of processing methods (Traditional against improved methods), the average crude protein, crude fat and Zinc were significantly different at the 5% confidence level (Table 3 and Appendix 20). However, the same methods showed no significant differences on means of moisture content, Calcium and Iron at the 5% confidence level (Table 3 and Appendix 20).

#### **4.2.1. Nutrient composition of dried anchovies as influenced by processing methods**

The observed average moisture content of hot smoked anchovies ( $15.3 \pm 1.2\%$ ) was lower than that of sun dried ( $17.8 \pm 0.5\%$ ) as indicated in Table 1 and Appendix 2. Arason (2003) reported that heating facilitates the extraction of water from a substance; therefore the lower moisture content in hot smoked anchovies was due to higher smoking temperature removing most of the water from the fish. According to Jallow (1995), fish at 10 – 15% moisture content has a shelf-life of 3 – 9 months when stored properly. If that is the case then the moisture content of the hot smoked fish agrees with the results of this study.

**Table 1: Nutrient composition of dried anchovies as influenced by processing methods**

Processing Methods	Replications	Mean nutrients per g of dry matter of edible portions					
	(N)	%	%	% Crude	Calcium	Iron	Zinc (mg)
		Moisture	Crude	Protein	(mg)	(mg)	
		Content	Fat				
Hot Smoking	8	15.3	3.3	69.8	27.2	2.9	3.4
Sun drying	24	17.8	3.9	61.7	12.2	4.0	3.5
Total	32	17.2	3.7	63.7	16.0	3.7	3.4
<b>LSD (p≤0.05)</b>		<b>2.3485</b>	<b>0.7016</b>	<b>5.2121</b>	<b>12.6401</b>	<b>1.3096</b>	<b>1.2592</b>



Generally, both hot smoked and sun-dried anchovies are primarily preserved by controlling water activity and water content. This is because the growth and multiplication of spoilage microorganisms depend on the available water activity. Consequently, measurement of water content is a typical way of determining the quality and storage time of the processed products.

The crude protein formed the largest quantity of the dry matter where the mean values were 69.8% and 61.7% for hot-smoked and sun-dried anchovies, respectively (Table 1 and Appendix 2). This is in-line with Daramola *et al*, (2007) who reported that protein forms the largest quantity of dry matter in fish. There was a reduction in the percentage of crude protein of the processed anchovies during the period of storage. The crude protein content for hot smoked products changed from 77% to 56.2%. In the present study, the crude protein content for sun-dried products decreased from 72.1% to 50.7% (Appendix 1).

The observed value of crude fat for sun-dried specimens (4.47%) was higher than that of hot-smoked samples (3.35%), though the difference between the two specimens was not significant at the 5% confidence level (Table 1 and Appendix 2). The reason behind the difference is application of higher temperature in hot-smoking than that of sun-drying of the anchovies. For the hot-smoked fish products the mean levels of calcium, iron and zinc were 27.2 mg/g, 2.9 mg/g and 3.4 mg/g, respectively. On the other hand, the levels of calcium, iron and zinc for the sun-dried products were 12.2 mg/g, 4.0 mg/g and 3.5 mg/g, respectively.

The difference between each of the mineral content in sun-dried and hot-smoked anchovies was caused by the way these two products prepared. The sundried anchovies prepared traditionally through boiling the fish for longer period of time than those prepared in improved manner. Consequently, more minerals were lost in sun-dried anchovies than those in hot-smoked anchovies. According to Foods Safety and Standards Authority of India (2010), cooking the food in water results in some losses of minerals since many minerals have significant solubility in water. Otherwise, the fresh fish were frozen during and then thawed prior processing by improved methods. This practice could lead to loss of minerals through leaching and or physical separation.

#### **4.2.2. Nutrient composition of dried anchovies as influenced by packaging aspects**

In case of packaging aspects, the observed moisture content of packaged dried anchovies (17%) was lower than that of un-packaged (17.3%) although, this difference was statistically insignificant at the 5% confidence level (Table 2 and Appendix 11). The slight difference could be contributed by the gain of moisture from the atmosphere by the un-packaged anchovies. The mean crude protein for packaged anchovies was 63.3% and the crude protein content changed from 75.2% to 53.6% (Table 2 and Appendix 10).

**Table 2: Nutrient composition of dried anchovies as influenced by packaging aspects**

<b>Packaging aspect</b>	<b>Replications</b>	<b>Mean nutrients per g of dry matter of edible portions</b>					
	<b>(N)</b>	<b>% Moisture Content</b>	<b>% Crude Fat</b>	<b>% Crude Protein</b>	<b>Calcium (mg)</b>	<b>Iron (mg)</b>	<b>Zinc (mg)</b>
Packaged	16	17.0	3.7	63.3	18.6	3.9	3.5
Un-packaged	16	17.3	3.8	64.1	13.3	3.6	3.4
Total	32	17.2	3.7	63.7	16.0	3.7	3.4
<b>LCD (p≤0.05)</b>		<b>2.1851</b>	<b>0.6311</b>	<b>5.2013</b>	<b>11.8075</b>	<b>1.1807</b>	<b>1.0891</b>

The crude protein value for un-packaged products changed from 77% to 50.7% and the mean crude protein value was 64.1%. The decreases in protein content could be due to gradual degradation of the initial crude protein to more volatile products such as total volatile bases (TVB), hydrogen sulphide and ammonia (Eyo, 2001).

Otherwise, the results for packaging aspects revealed that the mean value of crude fat for the packaged products (3.7%) was lower than that of un-packaged (3.8%). The difference between the two values was also insignificant at 5% confidence level. The difference in crude fat could be caused by the formation of water vapour in packaged anchovies which might lead to the physical separation of fat from the product to the packaging materials and consequently the crude fat content decreased. According to Srinivasa *et al.* (1998), polyethylene bags have disadvantages of high water vapour and gas transmission rates.

The results for packaged products revealed the average levels of calcium, iron and zinc as 18.6 mg/g, 3.9 mg/g and 3.5 mg/g, respectively. The un-packaged products indicated the mean levels of calcium, iron and zinc as 13.3 mg/g, 3.6 mg/g and 3.4 mg/g, respectively. The difference in specific mineral contents could be related to moisture loss induced by packaging of anchovies. According to Akintola *et al.* (2013), mineral content increases as the moisture content decreases.

#### **4.2.3. Nutrient composition of dried anchovies as influenced by categories of processing methods**

Regarding the categories of processing methods, the mean moisture content of improved methods (16.9%) was lower than that of traditional methods (17.5%)

although this difference was also statistically insignificant (Table 3 and Appendix 20). The mean crude protein levels of products processed by improved and traditional methods were 66.6% and 60.8%, respectively. The crude protein levels for the improved and traditional methods changed from 77% to 56.2% and from 72.1% to 50.7%, respectively. This observation could be caused by application of high temperatures in the improved processing methods of anchovies. The hot-smoking and sun-drying of anchovies using Altona kiln and solar tent dryer, respectively involved applications of high temperatures which resulted into loss of large amount of moisture content as compared to that of traditional methods. Consequently, the protein content of anchovies processed by improved methods was more concentrated than that of anchovies processed by traditional methods. Akintola *et al.* (2013) reported that an increase in amount of the removed moisture from the product may cause an increase in crude protein content of that product.

The average crude fat content for improved methods (3.3%) was less than that of traditional methods (4.2%), and the difference of the two values was significant at the 5% confidence level. The difference in fat content between products dried by traditional and improved methods could be contributed by the difference in temperatures. The temperature applied in the improved methods was higher than that of traditional methods. Consequently, the rate of fat degradation for the case of improved methods was higher than that of traditional methods, hence the low value for mean crude fat observed. According to The World's healthiest Foods (2014), factors which cause an oil to become rancid are oxygen, heat and light. The lipid in fish tissue contains poly-unsaturated fatty acids (PUFA) which can be oxidized to

products such as peroxides, aldehydes, ketones and the free fatty acids, eventually the lipid content is reduced (Horner, 1992).

**Table 3: Nutrient composition of dried anchovies as influenced by categories of processing methods**

Categories of Processing Methods	Replications (N)	Mean nutrients per g of dry matter of edible portions					
		% Moisture Content	% Crude Fat	% Crude Protein	Calcium (mg)	Iron (mg)	Zinc (mg)
Improved	16	16.9	3.3	66.6	17.2	4.2	4.0
Traditional	16	17.5	4.2	60.8	14.7	3.3	2.9
Total	32	17.2	3.7	63.7	16.0	3.7	3.5
<b>LSD (p≤0.05)</b>		<b>2.1772</b>	<b>0.537</b>	<b>4.7417</b>	<b>11.9310</b>	<b>1.1311</b>	<b>1.001</b>

Fish oil has been found to be more liable to spoilage than other oils due to their greater number of unsaturated fatty acids (Eyo, 1993).

For the case of improved processing methods, the mean levels of calcium, iron and zinc were 17.2 mg/g, 4.2 mg/g and 4.0 mg/g, respectively. The mean levels of calcium, iron and zinc for traditional processing methods were 14.7 mg/g, 3.3 mg/g and 2.9 mg/g, respectively. The mean values of Calcium and Iron were not significant from each other at the 5% confidence level. The micronutrient profiles of dried anchovies investigated in this study indicated that the fish products can contribute to nutrition security of low income populations that depend on these products as their major source of micronutrients. Although this study only focused on iron, calcium and zinc, the high levels of these nutrients suggests that the fish products are a good source of other micronutrients that are expected to be high in fish. This is in-line with the report by Kabahenda *et al.*, (2009) that *mukenne* (*Rastrineobola argentea*) products have high levels of calcium, iron and zinc which indicate the high levels of other micronutrients.

#### **4.3. Protein-bioavailability of Dried Anchovies**

The results for analyses of protein digestibility of dried anchovies are shown in Table 4. All three practices (processing methods, packaging aspects and categories of processing methods) had statistically no significant difference on protein digestibility of processed anchovies at the 5% confidence level (Appendices 9, 18 and 27).



**Table 4: Protein-digestibility of the dried anchovies during the fourth week as influenced by processing methods**

		<b>Replications</b>	<b>Mean protein-digestibility (%)</b>
		<b>(N)</b>	
<b>Processing Methods</b>	Hot smoking	2	73.8
	Sun drying	6	73.8
<b>Packaging aspects</b>	Packaged	4	73.8
	Un-packaged	4	73.8
<b>Categories of Processing Methods</b>	Improved	4	73.8
	Traditional	4	73.8
	Total	24	73.8
	<b>LSD (p≤0.05)</b>		<b>0</b>

#### **4.3.1 Protein-bioavailability of dried anchovies as influenced by processing methods and storage time**

The results of protein-digestibility, during the fourth week of storage, in all the three aspects of processing methods indicated that there was no significant difference ( $p > 0.05$ ). They had the same mean protein-digestibility value (73.8%) as indicated in Table 4. This implied that, provided there were no other factors which impaired protein-digestibility by the body, amount of protein available to the body depended on the nutrient density. Intawongse and Dean (2006) reported that nutrient bioavailability can be affected by various factors including the type and/or composition of food (e.g. anti-nutritional factors, components interactions), different processing/cooking procedures and gastrointestinal conditions. According to El and Kavas (1996), the protein-digestibility decreases as a result of complex chemical (cross-linking) reactions, such as protein interactions or protein-fat interactions when food is boiled at high temperatures. Given this fact it is expected to have higher protein-digestibility value in sun-dried anchovies than that of hot-smoked anchovies.

Deterioration of protein by any spoilage factor such as microbes and high temperature may lead to decrease in protein-density. Consequently, a consumer of the fish products does not get the required amount of protein. If the consumer depends on such kind of the fish product as the only source of animal protein, he/she may suffer from under-nutrition.

## CHAPTER FIVE

### 5.0 CONCLUSIONS AND RECOMMENDATIONS

#### 5.1 Conclusions

Although nutritional quality of the dried fish could predict the amount of nutrients found in the product, protein–bioavailability assessment is the more reliable method of determining the amount of nutrients expected to be available to the human body. All parameters in this study had been attained by using chemical methods. These methods showed reliable measures of freshness or degree of spoilage of the product as well as nutrient-bioavailability.

Therefore, based on chemical methods of assessment it was observed that the fish treated in all three different methods (processing, packaging and categories) underwent loss in nutritional quality. In spite of the observed nutritional loss, the fish products indicated to be of suitable nutritional value, under natural condition, within the four weeks of experiments. This implied that the processed fish products stored at ambient temperatures should be consumed within four weeks. In this case, the cost of storage was low since there was no special temperature regulator.

Despite the fact that the fish products were physically firm, precautions should be taken in consuming fish stored on open shelf for very long time, such fish could harbour microbial cells. A longer storage period could be attained under improved storage conditions, though more expensive. The microbial contamination could lead to fish spoilage and eventually decrease in nutrient density of the fish. Consequently,

consumers do not absorb enough nutrients to cover their needs for energy and growth, or to maintain a healthy immune system. In order to meet this strategy, number of preservative factors should furthermore increase so as to avoid microbial contamination. The nutrient density of fish products due to improved methods was higher than that of traditional methods, in terms of protein content. Based on moisture contents of anchovies processed by improved methods, their shelf-life could be extended for more than a month.

## **5.2 Recommendations**

Based on the results of this study, the following recommendations are made:

- In anchovies drying, solar tent dryer should be used instead of open sun-drying in order to reduce the microbial contamination from the surrounding atmosphere.
- Moisture content of less than 10% should be attained during processing and maintained in stored dried fish to reduce microbial growth.
- Cooking fish with lemon or tamarind or eating fruits and vegetables rich in Vitamin C during the same meal will help the body absorb the iron in fish.
- The Altona kiln oven should be used in hot smoking of fish in order to dry the fish throughout the year, even during the rainy season.

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## APPENDICES

**Appendix 1: Descriptive statistics Table for nutrient composition and protein-bioavailability of dried anchovies as influenced by processing methods**

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
%AV MC Hot smoking	8	15.302	3.3817	1.1956	12.475	18.130	11.6	21.1
Sun drying	24	17.796	2.6207	.5349	16.689	18.902	13.6	22.0
Total	32	17.172	2.9801	.5268	16.098	18.247	11.6	22.0
%AV CP Hot smoking	8	69.771	6.3111	2.2313	64.495	75.047	56.2	77.0
Sun drying	24	61.717	6.2329	1.2723	59.086	64.349	50.7	72.1
Total	32	63.731	7.0973	1.2546	61.172	66.290	50.7	77.0

%AV CF	Hot smoking	8	3.346	.6739	.2383	2.782	3.909	2.4	4.6
	Sun drying	24	3.883	.8862	.1809	3.509	4.257	2.1	5.3
	Total	32	3.749	.8609	.1522	3.438	4.059	2.1	5.3
Calcium (mg/g)	Hot smoking	8	27.1862	21.76096	7.69366	8.9936	45.3789	3.34	56.21
	Sun drying	24	12.2144	12.47649	2.54675	6.9460	17.4827	2.80	51.81
	Total	32	15.9573	16.30354	2.88209	10.0793	21.8354	2.80	56.21
Zinc (mg/g)	Hot smoking	8	3.3975	1.81669	.64230	1.8787	4.9163	.62	4.96
	Sun drying	24	3.4725	1.40387	.28656	2.8797	4.0653	.67	5.65
	Total	32	3.4537	1.48613	.26271	2.9179	3.9896	.62	5.65
Iron (mg/g)	Hot smoking	8	2.9406	1.45118	.51307	1.7274	4.1538	1.23	5.20
	Sun drying	24	4.0042	1.60520	.32766	3.3263	4.6820	1.61	7.86
	Total	32	3.7383	1.61437	.28538	3.1562	4.3203	1.23	7.86

Protein	Hot	2	73.7550	.00707	.00500	73.6915	73.8185	73.75	73.76
digestibility	smoking								
	Sun	6	73.7600	.01897	.00775	73.7401	73.7799	73.74	73.79
	drying								
	Total	8	73.7587	.01642	.00581	73.7450	73.7725	73.74	73.79

**Appendix 2: One way ANOVA Table for nutrient composition and protein-bioavailability analyses of dried anchovies as influenced by processing methods**

		Sum of Squares	df	Mean Square	F	P value
%AV MC	Between Groups	37.298	1	37.298	4.701	.038
	Within Groups	238.015	30	7.934		
	Total	275.313	31			
%AV CP	Between Groups	389.171	1	389.171	9.959	.004
	Within Groups	1172.329	30	39.078		
	Total	1561.500	31			
%AV CF	Between Groups	1.734	1	1.734	2.449	.128
	Within Groups	21.243	30	.708		
	Total	22.977	31			

Calcium (mg/g)	Between	1344.942	1	1344.942	5.852	.022
	Groups					
	Within	6895.022	30	229.834		
	Groups					
	Total	8239.965	31			
Zinc (mg/g)	Between	.034	1	.034	.015	.904
	Groups					
	Within	68.432	30	2.281		
	Groups					
	Total	68.466	31			
Iron (mg/g)	Between	6.787	1	6.787	2.751	.108
	Groups					
	Within	74.005	30	2.467		
	Groups					
	Total	80.792	31			
Protein digestibility	Between	.000	1	.000	.122	.739
	Groups					
	Within	.002	6	.000		
	Groups					
	Total	.002	7			

**Appendix 3: The least significant difference (lsd) at the 5% confidence level for moisture content (AV\_MC)**

Minimum least significant difference	2.348
Average least significant difference	2.348
Maximum least significant difference	2.348

The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$

**Appendix 4: The least significant difference (lsd) at the 5% confidence level for crude protein (AV\_CP)**

Minimum least significant difference	5.212
Average least significant difference	5.212
Maximum least significant difference	5.212

The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$

**Appendix 5: The least significant difference (lsd) at the 5% confidence level for crude fat (AV\_CF)**

Minimum least significant difference	0.7016
Average least significant difference	0.7016

Maximum least significant difference	0.7016
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The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$

**Appendix 6: The least significant difference (lsd) at the 5% confidence level for Calcium content (Calcium)**

Minimum least significant difference	12.64
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Average least significant difference	12.64
--------------------------------------	-------

Maximum least significant difference	12.64
--------------------------------------	-------

The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$

**Appendix 7: The least significant difference (lsd) at the 5% confidence level for Zinc content (Zinc)**

Minimum least significant difference	1.259
--------------------------------------	-------

Average least significant difference	1.259
--------------------------------------	-------

Maximum least significant difference	1.259
--------------------------------------	-------

The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$

**Appendix 8: The least significant difference (lsd) at the 5% confidence level for  
Iron content (Iron)**

Minimum least significant difference	1.310
Average least significant difference	1.310
Maximum least significant difference	1.310

The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$

**Appendix 9: The least significant difference (lsd) at the 5% confidence level for  
protein-digestibility (PD)**

Minimum least significant difference	0.03508
Average least significant difference	0.03508
Maximum least significant difference	0.03508

The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$



**Appendix 10: Descriptive statistics Table for Nutrient composition and protein-bioavailability of dried anchovies as influenced by packaging aspects**

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
%AV MC	Packaged	16	17.038	2.6977	.6744	15.600	18.475	13.5	21.2
	Un-packaged	16	17.307	3.3223	.8306	15.537	19.078	11.6	22.0
	Total	32	17.172	2.9801	.5268	16.098	18.247	11.6	22.0
%AV CP	Packaged	16	63.343	6.9211	1.7303	59.655	67.031	53.6	75.2
	Un-packaged	16	64.119	7.4750	1.8688	60.136	68.102	50.7	77.0
	Total	32	63.731	7.0973	1.2546	61.172	66.290	50.7	77.0
%AV CF	Packaged	16	3.701	.7812	.1953	3.285	4.118	2.4	5.0
	Un-packaged	16	3.796	.9575	.2394	3.286	4.306	2.1	5.3

	Total	32	3.749	.8609	.1522	3.438	4.059	2.1	5.3
Calcium (mg/g)	Packaged	16	18.5662	18.96390	4.74098	8.4611	28.6714	3.89	56.21
	Un- packaged	16	13.3484	13.23560	3.30890	6.2957	20.4012	2.80	48.16
	Total	32	15.9573	16.30354	2.88209	10.0793	21.8354	2.80	56.21
Zinc (mg/g)	Packaged	16	3.5334	1.61124	.40281	2.6749	4.3920	.62	5.65
	Un- packaged	16	3.3741	1.39812	.34953	2.6291	4.1191	.72	4.94
	Total	32	3.4537	1.48613	.26271	2.9179	3.9896	.62	5.65
Iron (mg/g)	Packaged	16	3.8734	1.88773	.47193	2.8675	4.8793	1.23	7.86
	Un- packaged	16	3.6031	1.33552	.33388	2.8915	4.3148	1.61	6.53
	Total	32	3.7383	1.61437	.28538	3.1562	4.3203	1.23	7.86
Protein digestibi lity	Packaged	4	73.7550	.01291	.00645	73.7345	73.7755	73.74	73.77
	Un- packaged	4	73.7625	.02062	.01031	73.7297	73.7953	73.74	73.79
	Total	8	73.7587	.01642	.00581	73.7450	73.7725	73.74	73.79

**Appendix 11: One way ANOVA Table for nutrient composition and protein-bioavailability analyses of dried anchovies as influenced by packaging aspects**

		Sum of Squares	df	Mean Square	F	Sig. (LSD)
%AV MC	Between Groups	.582	1	.582	.064	.803
	Within Groups	274.731	30	9.158		
	Total	275.313	31			
%AV CP	Between Groups	4.824	1	4.824	.093	.763
	Within Groups	1556.676	30	51.889		
	Total	1561.500	31			
%AV CF	Between Groups	.072	1	.072	.094	.761
	Within Groups	22.905	30	.764		
	Total	22.977	31			

Calcium (mg/g)	Between Groups	217.805	1	217.805	.815	.374
	Within Groups	8022.160	30	267.405		
	Total	8239.965	31			
Zinc (mg/g)	Between Groups	.203	1	.203	.089	.767
	Within Groups	68.263	30	2.275		
	Total	68.466	31			
Iron (mg/g)	Between Groups	.585	1	.585	.219	.643
	Within Groups	80.207	30	2.674		
	Total	80.792	31			
Protein digestibility	Between Groups	.000	1	.000	.380	.560
	Within Groups	.002	6	.000		
	Total	.002	7			

**Appendix 12: The least significant difference (lsd) at the 5% confidence level for moisture content (AV\_MC)**

Table	Pack_aspect
rep.	16
d.f.	30
l.s.d.	2.185

The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$

**Appendix 13: The least significant difference (lsd) at the 5% confidence level for crude protein (AV\_CP)**

Table	Pack_aspect
rep.	16
d.f.	30
l.s.d.	5.20

The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$

**Appendix 14: The least significant difference (lsd) at the 5% confidence level for crude fat (AV\_CF)**

Table	Pack_aspect
rep.	16

d.f. 30

l.s.d. 0.631

The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$

**Appendix 15: The least significant difference (lsd) at the 5% confidence level for Calcium content (Calcium)**

Table Pack\_aspect

rep. 16

d.f. 30

l.s.d. 11.81

The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$

**Appendix 16: The least significant difference (lsd) at the 5% confidence level for Zinc content (Zinc)**

Table Pack\_aspect

rep. 16

d.f. 30

l.s.d. 1.089

The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$

**Appendix 17: The least significant difference (lsd) at the 5% confidence level for  
Iron content (Iron)**

Table	Pack_aspect
rep.	16
d.f.	30
l.s.d.	1.181

The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$

**Appendix 18: The least significant difference (lsd) at the 5% confidence level for  
protein-digestibility (PD)**

Table	Pack_aspect
rep.	4
d.f.	6
l.s.d.	0.02976

The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$

**Appendix 19: Descriptive statistics Table for nutrient composition and protein digestibility of dried anchovies as influenced by categories of processing methods (intervention)**

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
%AV MC	Improved	16	16.890	3.2120	.8030	15.178	18.601	11.6	21.3
	Traditional	16	17.455	2.8047	.7012	15.961	18.950	13.6	22.0
	Total	32	17.172	2.9801	.5268	16.098	18.247	11.6	22.0
%AV CP	Improved	16	66.624	6.0185	1.5046	63.417	69.831	56.2	77.0
	Traditional	16	60.838	7.0728	1.7682	57.069	64.607	50.7	72.1
	Total	32	63.731	7.0973	1.2546	61.172	66.290	50.7	77.0
%AV CF	Improved	16	3.301	.6937	.1734	2.932	3.671	2.1	4.6
	Traditional	16	4.196	.7898	.1975	3.775	4.617	2.8	5.3
	Total	32	3.749	.8609	.1522	3.438	4.059	2.1	5.3
Calcium (mg/g)	Improved	16	17.1962	18.23659	4.55915	7.4787	26.9138	2.80	56.21
	Traditional	16	14.7184	14.61107	3.65277	6.9327	22.5041	2.96	51.81
	Total	32	15.9573	16.30354	2.88209	10.0793	21.8354	2.80	56.21
Zinc	Improved	16	4.0350	1.46496	.36624	3.2544	4.8156	.62	5.65



(mg/g)	Traditional	16	2.8725	1.30289	.32572	2.1782	3.5668	.67	4.34
	Total	32	3.4537	1.48613	.26271	2.9179	3.9896	.62	5.65
Iron (mg/g)	Improved	16	4.2119	2.00184	.50046	3.1452	5.2786	1.23	7.86
	Traditional	16	3.2647	.94883	.23721	2.7591	3.7703	1.61	5.14
	Total	32	3.7383	1.61437	.28538	3.1562	4.3203	1.23	7.86
Protein digestibility	Improved	4	73.7565	.00957	.00479	73.7373	73.7677	73.74	73.76
	Traditional	4	73.7640	.02082	.01041	73.7319	73.7981	73.74	73.79
	Total	8	73.7603	.01642	.00581	73.7450	73.7725	73.74	73.79

**Appendix 20: One way ANOVA Table for nutrient composition and protein-bioavailability analyses of dried anchovies as influenced by categories of processing methods (intervention)**

## ANOVA

		Sum of Squares	df	Mean Square	F	Sig.(LSD)
%AV MC	Between Groups	2.561	1	2.561	.282	.600
	Within Groups	272.752	30	9.092		
	Total	275.313	31			
%AV CP	Between Groups	267.794	1	267.794	6.210	.018
	Within Groups	1293.706	30	43.124		
	Total	1561.500	31			
%AV CF	Between Groups	6.401	1	6.401	11.585	.002
	Within Groups	16.576	30	.553		
	Total	22.977	31			

Calcium (mg/g)	Between Groups	49.116	1	49.116	.180	.674
	Within Groups	8190.848	30	273.028		
	Total	8239.965	31			
Zinc (mg/g)	Between Groups	10.811	1	10.811	5.626	.024
	Within Groups	57.655	30	1.922		
	Total	68.466	31			
Iron (mg/g)	Between Groups	7.177	1	7.177	2.925	.098
	Within Groups	73.614	30	2.454		
	Total	80.792	31			
Protein digestibility	Between Groups	.000	1	.000	1.190	.317
	Within Groups	.002	6	.000		
	Total	.002	7			

**Appendix 21: The least significant difference (lsd) at the 5% confidence level for moisture content (AV\_MC)**

Table	Intervention
rep.	16
d.f.	30
l.s.d.	2.177

The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$

**Appendix 22: The least significant difference (lsd) at the 5% confidence level for crude protein (AV\_CP)**

Table	Intervention
rep.	16
d.f.	30
l.s.d.	4.74

The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$

**Appendix 23: The least significant difference (lsd) at the 5% confidence level for crude fat (AV\_CF)**

Table	Intervention
rep.	16
d.f.	30
l.s.d.	0.537

The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$

**Appendix 24: The least significant difference (lsd) at the 5% confidence level for Calcium content (Calcium)**

Table	Intervention
rep.	16
d.f.	30
l.s.d.	11.93

The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$

**Appendix 25: The least significant difference (lsd) at the 5% confidence level for Zinc content (Zinc)**

Table	Intervention
rep.	16
d.f.	30
l.s.d.	1.001

The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$

**Appendix 26: The least significant difference (lsd) at the 5% confidence level for  
Iron content (Iron)**

Table	Intervention
rep.	16
d.f.	30
l.s.d.	1.131

The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$

**Appendix 27: The least significant difference (lsd) at the 5% confidence level for  
Protein digestibility (PD)**

Table	Intervention
rep.	4
d.f.	6
l.s.d.	0.02803

The LSD test declares the difference between means  $\hat{Y}_1$  and  $\hat{Y}_2$  of treatments  $T_1$  and  $T_2$  to be significant when:

$$|\hat{Y}_1 - \hat{Y}_2| > \text{LSD}$$