KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

USING MACKEREL (*SCOMBEROMORUS TRITOR)* AND CATFISH (*CLARIAS GARIEPINUS)* IN FRANKFURTER-TYPE SAUSAGES

A THESIS SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE COLLEGE OF AGRICULTURE AND NATURAL RESOURCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER OF PHILOSOPHY (MEAT SCIENCE)

BY

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DECLARATION

I, Theresah Nkrumah, hereby declare that the work presented in this thesis is the result of my own hard work and no such previous application for a degree in this University or elsewhere has the same work being presented.

All sources of information have been acknowledged by reference to authors.

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ABSTRACT

In this study essential amino acids, minerals, proximate composition, pH, water holding capacity, and microbiological safety of meats (Mackerel, Catfish and Pork) and their corresponding sausages were determined. Amino acids were determined using high performance liquid chromatography (HPLC) Mg^{2+}, Ca^{2+}, K^{+}, NH_4^{+} and Na^{+} were determined by Cadmium.mtw and ASUP5–100 marvin.mtw was used for determining the anions (Fl^{-}, Cl^{-}, $N0_3^{-}$, $S0_4^{2-}$ and $P0_4^{3-}$). Proximate compositions were determined using the methods recommended by Association of Official Analytical Chemists. Sensory evaluation was also conducted in order to evaluate consumer acceptability of the sausages. Hedonic scale rating was used to score sensory attributes (appearance, taste, texture, juiciness, flavour, mouthfeel, and overall acceptability) of the sausages. Total Viable Counts and isolation of *Escherichia coli* were performed using methods of the International Commission on Microbial Specification for Foods (ICMSF). Catfish sausages recorded higher protein levels (18.86% to12.52%) and were low in fat (10.45% to10.87%) compared to pork and mackerel sausages (19.06 to 19.48 and 23.21 to 23.39) respectively. Pork and catfish sausages were able to hold more water than mackerel sausage in both emulsion and sausages. The pH of mackerel and catfish were higher ($P<0.05$) than pork in both emulsion and sausages. Aspartic acid, glutamic acid, serine, arginine, methionine, threonine and leucine levels in pork were higher than those of raw fish (catfish and mackerel). Though raw pork recorded higher values in most of the essential amino acids than raw fish (mackerel and catfish), sausages from mackerel, and catfish recorded higher values, compared to pork sausages. Raw mackerel recorded the highest values in most of the ions determined (except ammonium, potassium and magnesium) followed by catfish and pork, however mackerel and pork sausages without corn starch performed better numerically in the ion deposits. Most of the sensory panelists

preferred pork sausages to fish sausages. Very high scores were obtained for the overall product acceptability and for most of the individual sensory attributes.

Overall product acceptability was significantly lower ($p<0.05$) in mackerel sausage. Total Viable Count (TVC) recorded was within the accepted limits (10^6 and 10^7cfu/g) for fish and pork respectively. *E.coli* was not detected in any of treatments during frozen storage for 6 weeks. It was concluded that catfish frankfurter has high nutritive value because it contained less fat with high crude protein and has high cooking yield.

TABLE OF CONTENTS

DECLARATION ii

ABSTRACT iii

TABLE OF CONTENTS v

LIST OF TABLES viii

LIST OF FIGURES ix

LIST OF PLATES x

LIST OF ABBREVIATION xi

DEDICATION xii

ACKNOWLEDGEMENT xiii

CHAPTER ONE 1

1.0 INTRODUCTION 1

CHAPTER TWO 5

2.0 LITERATURE REVIEW 5

2.1 Protein in Human Food 5

2.1.1 Protein Requirement of Humans 6

2.1.2 Protein Consumption 8

2.2 Amino Acids in Foods 12

2.3 Fish as Protein Source 13

2.3.1 Other Benefits of Fish 14

2.3.2 Problems with Fish as Food 15

2.4 Microorganisms in Meat 18

2.5 Fish Processing 19

2.5.1 Modern Fish Processing 21

2.5.1.1 Canning 22

2.5.1.2 Mincing 22
2.5.2 Conventional / Traditional Methods of Processing Fish in Ghana 23
2.5.2.1 Salting 23
2.5.2.2 Smoking 25
2.5.2.3 Drying 27
2.5.2.4 Freezing 30
2.6 Modern Methods of Processing Meat 31
2.7 Sausages and their Classification 32
2.7.1 Fish Sausages 32
2.8 Ions (Minerals) in Meat 33
2.9 Water Holding Capacity (WHC) of Meat 36
2.10 Inferences from Literature 37

CHAPTER THREE 39
3.0 MATERIALS AND METHODS 39
3.1 Experimental Location and Design 39
3.2 Source of Raw Materials and Initial Preparation 39
3.3 Fish Sausage Preparation 40
3.3.1 Mincing 40
3.3.2 Sausage Formulation 41
3.4. Parameters Measured 42
3.4.1 Physicochemical characteristics 42
3.4.2 Amino acids and Ions determination 42
3.4.3 Microbial safety [Total Viable Count (TVC) and Isolation of *Escherichia coli*] 43
3.3.4 Sensory Attributes 43
3.4 Statistical Analysis 44

CHAPTER FOUR 45
4.0 RESULTS AND DISCUSSION 45
4.1 Acidity (pH) and Water Holding Capacity (WHC) 45
4.2 Cooking Yield 47
4. 3 Cost of Production 48
4.4. Proximate Composition 48
4.5. Sensory Parameters 51
4. 6 Essential Amino Acid Contents of Fish (Mackerel and Catfish) and Pork 53
4.7. Cations and Anions in Meats and Sausages 54
4.8. Microbial Counts and Isolation of *E. coli* 57
CHAPTER FIVE 62
5.0 CONCLUSIONS AND RECOMMENDATIONS 62
5.1 Conclusions..... 62
5.2 Recommendation 63
6.0 REFERENCES 64
7.0 APPENDICES 77

LIST OF TABLES

Table 2.0. Protein requirement standards for humans 7
Table 2.1: Protein requirements in human (g/kg per day) 7
Table 2.2: Some protein quality foods 12
Table 2.3 Essential amino acids in various animal protein sources 13
Table 3.0 Ingredient used in sausage formulation 41
Table 4.0 Acidity (pH), Water Holding Capacity and Cooking Yield 47
Table 4.1 Cost of ingredients used in sausage production..... 48
Table 4.2 Proximate composition of sausage used (As - In) during storage 49

Table 4.3 Sensory attributes of pork and fish frankfurters 52

Table 4.4.1 Essential Amino Acids Content of Fish (Mackerel and Catfish) and Pork ... 53

Table 4.4.2 Essential Amino acid contents of pork and fish sausages 54

Table 4.5. Total Viable Count (TVC) for minced meat 58

Table 4.5.1 Total Viable Count (TVC) for Raw Meat batter 58

Table 4.5.2 Total Viable Count (TVC) of sausages during frozen storage 59

Table 4.6.0 Isolation of organism (*E. coli)* in minced meat 59

Table 4.6.1 Isolation of organism *(E. coli)* in raw batter 60

Table 4.6.2 Isolation of organism (*E. coli*) in frankfurter during frozen storage (week 1-week 3) 60

Table 4.6.3 Isolation of organism *(E. coli*) in frankfurter during frozen storage (week 4-week 6) 61

LIST OF FIGURES

Figure 2.0 Total protein supplies by continent and major food groups 10

Figure 2.1 Meat products grouped according to the processing technology applied 31

Figure 2.2 Average Total Body Content of Major Minerals (g) 35

Figure 3.4 Flow Chart for sausage production 42

Figure 4.1 Ions (ppm) in raw meat 55

Figure 4.2 Levels of Mg^{2+}, $N0_3^-$, Ca^{2+} and NH_4^+ determined in Sausages 56

Figure 4.2 Levels of Na^+, $P0_4^{3-}$, Cl^-, $S0_4^{2-}$, F^- and K^+ determined in Sausages 57

LIST OF PLATES

Plate 3.1: Skinning catfish 40

Plate 3.0: Evisceration and gutting of catfish 40

Plate 3.2: Deboning catfish 40

Plate 3.3: Deboning Mackerel 40

LIST OF ABBREVIATION

AOAC	Association of Official Analytical Chemist
C	Catfish
CFS	Catfish Frankfurter
CFU	Colony Forming Unit
CRD	Completely Randomised Design
FAO	Food and Agriculture Organization
GFSI	Global Food Security Index
HACCP	Hazard Analysis and Critical Control Point
HSPH	Havard School of Public Health
ICMSF	International Commission on Microbiological Specification on Food
IoM	Institute of Medicine
M	Mackerel
MFS	Mackerel Frankfurter
NAS	National Academy of Science
NPPC	National Pork Producers Council
P	Pork
PFS (+VE)	Pork Frankfurter without corn starch
PFS (-VE)	Pork Frankfurter with corn starch
SP	Sampling Point
TVC	Total Viable Count
UNO	United Nation Organization
USDA	United States Department of Agriculture
WHC	Water Holding capacity
WHO	World Health Organization

DEDICATION

This work is dedicated to my father Nana Kofi Nkrumah

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I might not know where life's road will take me, but walking with you, God, through this journey has given me strength to soldier on the through to the end. I thank you Lord.

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CHAPTER ONE

1.0 INTRODUCTION

According to the FAO (2013b), the world fish industry is experiencing rapid growth due to its increased consumption levels and the Ghanaian industry is also recording high consumption levels. The increasing awareness of fish as food is due to its nutritional value, health benefits as well as affordability (Sathivel *et al*., 2009). Nestel *et al.* (1999) observed that fish contains low lipids, high amounts of protein and higher water than beef, pork and chicken and is preferred to other white or red meats. Steffens (2006), indicated that the nutritional value of fish meat comprises of moisture, protein, vitamins, minerals and the caloric value. Moore *et al*. (1988), reported of appreciable amounts of essential amino acids in fish, low levels of saturated fat and cholesterol compared with other animal protein sources. According to USDA (2003) and Ocaño-Higuera *et al.* (2011) fish has higher essential amino acids compared to beef as well as adequate dietary levels of long chain polyunsaturated fatty acids, including eicosapentaenoic and docosahexaenoic acid, which are good for the human body.

Ghana has large fisheries resources however, little is known about the nutritional value of the fishes (Kagan, 1970). Moreover, the poor shelf quality of fish poses some challenges to consumers and the fish industry in general. Consumers only have the option of choosing from preserved fish products like cured/salted fish, smoked/dried fish, canned fish and chilled/frozen fish (Breisinger *et al.*, 2011). Oduor-Odote and Kazungu (2008), stated that farmers and producers on the other hand are at the risk of diverting fish into lower value processed products such as fish meal. This seems to hold through the years, although clear financial losses from "down-grading" are higher during the peak landing seasons. Value-added products help the growth of the fish industry worldwide. The development of value-

added fish products like fish sausages, fish balls, grilled fish, fish fingers, steaks, fish cakes, smoked fish products, cured fish, fish burger, fresh or cooked product, fish loaf and canned fish have increased the consumption of fish and fish products in countries like Egypt and Uganda (Per Capita consumption of 4 kg for Egypt and Uganda 8.8 kg) (Ven den Bosssche and Bernacsek, 1990). Kondaiah (2004), reported that value addition offers consumers with variety of protein sources.

The producer through value added products will help sustain demand for fish products, serve as means of preservation, enable easy transport and distributing to larger consumers, promote entrepreneurial ventures and employment opportunities as well as enhance efficient marketing of fish to earn reasonable returns. Value addition means additional activity or processes that alter the nature of a product from its natural form and thus, adding to its value at the time of sale (Oduor– Odote and Kazungu, 2008). A USDA (1999) report stated that, 68% of fish is sold as fillet with the remaining (32%) sold as steaks, nuggets, and value-added products. Also, 72% of the live fish weight is sold for human food while the remainder (28%) is waste, including skeletons (frames), accounting for 10.5% of the whole production (Dean, 1996) which is used for down - graded fish products. Tacon (1992) reported of some delicatessen fish products, like fish sausages, fish crisps, fish chips and other products from fish. The world fish industry has come out with processed or minced fish products like fish burgers, fingers and sausages, which offer consumers cooking convenience, health and nutritional benefits. OduorOdote and Kazungu (2008) and Tozer (2001), have also exploited and used Tilapia for other forms of food consumption such as fish cakes and fish fingers. According to Unit (2011), Ghana was able to increase its average per capita consumption (PCC) of fish from 1.4 kg to 3.2 kg annually; championed by the Ministry of Food and Agriculture.

This was to revive the conventional fish processing methods. Moreover, an attempt to increase per capita production and consumption was to open sections of retail outlets for the conventional processed fish products like smoked, salted, dried, chilled or frozen fish products. The rapid increase in the world's population and limited supply of available food resources as well as shortage in protein intake requires urgent measures to fully utilize all fish resources (Olatunde *et al*., 2012 as cited by Fakagawu, 2014). One approach according to Oduor-Odote and Kazungu (2008), is to reduce the loss that occurs in the post harvest sector and add value to raw fish. Among these post harvest losses are sometimes underutilized fishes in the Ghanaian market (Catfish, *Clarias gariepinus)* (Prein and Ofori, 1996). Catfish is lowly priced because it contains numerous bones coupled with flat big head and is mainly consumed by those below the poverty line (Anihouvi *et al*., 2012). Mackerel (*Scomberomorus tritor)* on the other hand is an imported fish, which is dominant in the Ghanaian market and is usually affordable to small and medium income earners.

Kondaiah (2004) reported that, producing quality value added fish products will help meet the requirements in the Post-World Trade Organization period by successfully facing global competition. The manufacturer adding value to fish will help reduce waste by processing fish after harvesting to prevent it from deteriorating (FAO, 2013). According to Pearson and Gillett (1999) the processing methods of fish add more value to the product. Fish sausage which is a product from the processing of raw fish offers consumers a variety of fish product which can be served for breakfast, brunch, dinner or as a snack. The flesh of fish is usually used for sausage production because of its muscle protein as well as its unique characteristic gel forming which acts as an emulsifying agent (Yada *et al*., 1995). Fish sausage refers to minced, chopped or comminuted seasoned fish products that may be cured, smoked, shaped, and heat treated. In many instances, the consumer cooks the

products prior to serving and products are consumed hot (Steffens, 2006). Fish sausage has all the quality characteristics as food owing to its appreciable amount of protein and reduced levels of fat. Fish sausage paste has decisive influence on quality factors such as texture, flavour, appearance and nutritive value. It is therefore essential to learn how to make better use of fish by converting it to other forms of food such as fish sausage, a form that will be appreciated and acceptable by consumers (Olapade and Karim, 2011).

The main objective of the study was therefore to produce two frankfurter-type sausages using Mackerel and Catfish.

The Specific objectives were to evaluate the quality of these products by assessing their

- physicochemical
- microbial
- sensory and
- nutritional characteristics

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Protein in Human Food

Handling energy and also protein needs connected with humans is a long-standing activity of the FAO and also WHO. As outlined by Marshall (2011), the most effective utilize pertaining to proteins is usually to repair and maintain body tissues as well as promote growth in the human body. Olatunde *et al*. (2012) reported that, as soon as the human body gets enough proteins, it helps to maintain normal body make up and function throughout the life-cycle and this also is essential for maintaining health. Proteins are extremely valuable components of food that guarantees absorption connected with nitrogen and also amino acids to the activity and also preservation of the extensive volume of various other protein systems. This vital amino acids present in the proteins as well as the scope to be able to which tend to be oxidized pertaining to proteins activity describes proteins excellent (Fagakawu, 2014).

Proteins is among the nutritional requirements together with carbohydrates, minerals, fat, nutritional vitamins, and also water which help in entire body development and growth. The World Health Organization and also FAO (2002) reported that the volume of protein that is needed to be used to attain the proper ratio, structure and function in the systems as it's nutritionally satisfactory eating habits makes it a requirement. The benefit connected with satisfactory proteins absorption complemented with active lifestyle helps promote healthy aging, bone health by increasing calcium absorption, negating previous evidence to the contrary and prevention of degenerative loss of skeletal muscle (Yada *et al.*, 1995).

2.1.1 Protein Requirement of Humans

Protein is the building block of human life and is essential for the growth of cells and tissue repair. It helps to build lean muscle mass and supports muscle recuperation, contributing

to improved bone health. Proteins control appetite by keeping an individual for longer period, it is essential for energy as well as help the immune system to function properly. Protein is vital for human survival, but that does not guarantee its abuse when eating protein foods. Recommended levels are required. According to Fakagawu (2014) protein requirement is the continuous intake of dietary protein that is adequate to achieve body composition at the energy balance and under conditions of reasonable physical activity as determined after brief period of adjustment to change in protein intake or consumption. Berman *et al.* (2011) indicated that, there is no one-size that fit all answers to the question how much protein is required per person daily. Part of the reason for the obvious lack of clarity is because our technical ability to make measurements has advanced and our understanding and awareness has developed (Fouillet *et al*., 2008). Though individual protein requirements depend on several variables; there are some baseline intakes that can serve as a foundation for different groups. These baselines are estimates of the requirements of individuals and groups of individuals (FAO/WHO/UN, 1985). Report by Marshal (2011) revealed that how much protein an individual needs depend on his or her age, body size, activity level and special needs, like growth, maintenance, pregnancy and lactation. Children, pregnant women and lactating mothers need more protein in the body weight than adults because they are growing and building new body tissues (NAS, 2002). The IoM (2002) recommends a minimum of 0.8 - 1.0 grams of protein daily for every kilogram of body weight for adults. The minimum protein requirement for pregnant mothers according to the IoM (2002) is about 10 grams more than the requirement standards for other individuals. Table 2.0 shows the daily protein requirement for humans.

Table 2.0. Protein requirement standards for humans

Gender	**Age**	**Daily protein requirement (g)**
Children	0-6 months	9.3

Children	7 months -1 year	13.5
Children	1-3 years	13
Children	4- 8 years	19
Children	9- 13 years	34
Men	14- 18 years	52
Men	19+	56
Female	14+	46
Pregnant and lactation women	-	+ 25 to 71

Source: (NAS, 2002).

A joint report from WHO/UNU expert consultation (FAO, 1985**)** has also provided baseline data for the daily protein requirement for maintenance and growth of the body (Table 2.1).

Table 2.1: Protein requirements in human (g/kg per day)

Age (Years)	Maintenance	Growth [c]
0.5	0.66	0.46
1-2	0.66	0.20
3-10	0.66	0.07
11-14	0.66	0.07
15-18	0.66	0.04
>18	0.66	0

[c] *Calculated as average values for the age range growth adjusted for protein utilization of 58%*

Source: FAO (2007).

According to data on hand, the effect of an increase in protein intake on protein renewal is complex and does not allow a conclusion to be reached regarding its use as a protein requirement marker (Fouillet *et al.*, 2008; Harber *et al.*, 2005; Morens *et al.*, 2003; Forslund *et al.*, 1998; Millward *et al.*, 1994; Price *et al*., 1994). Lagiou *et al.* (2011) reported that, in every healthy individual, protein intake increase from 20 to 25 percent of calories reduces the risk of heart disease. Moreover, higher protein intake can also be

helpful for weight loss as well as reduced calorie diet, although long-term evidence of their effectiveness is still under review.

2.1.2 Protein Consumption

Protein is essential to the human body. It plays a vital role in the body by forming the foundation of muscles, hair, nails and collagen that holds the body together. Proteins are also required for the synthesis of a range of metabolic products, including neurotransmitters, thyroid hormones and haem, found in red blood cells (Hermann, 2011).

Unit (2011) found that protein consumption in the Ghanaian diet is low. This insinuates that Ghanaians' food intake does not match the body's requirement of having a balanced diet thus, resulting in malnutrition. FAO (1985) report based on state-of-the-art epidemiological evidence by the Ghana Health Service showed that deaths (forty percent 40%) that occur in growing children before the age of five are partially due to low protein intake, making it the single most important cause of child mortality. Ghana is well known as a fish eating nation and fish is generally enjoying a high consumer preference all over the country. According to the 2007 Budget Statement, the country's total annual fish requirement will be estimated at 720,000 metric tons (mt), while annual production averages 400,000mt. This leaves an annual deficit of 320,000mt which is made up through the importation of US$200 million worth of fish into the country.

The Ghanaian eating habits largely relies on starchy roots (cassava, yams), fruit (plantain) and cereals (maize, rice). Starchy roots and cereals still provide almost three quarters of the dietary energy and variety of the diet remains low (FAO, 2002). The dietary contribution meets population energy requirements, but the contribution of protein and lipids in the dietary energy supply is lower than recommendations. The amino acids present in the protein assist in tissue synthesis through growth particularly in infants, children and

adolescence. FAO (2001) advises increase protein intake since it helps maintain a healthy body weight. According to IoM (2002), proteins form important constituents of hormones. A deficit of protein in the body could mean trouble; this is so because protein is important for many bodily features through developing and also keeping muscle tissue as well as bone to keeping cells in the appropriate working order. Wu *et al*. (2001) reported that protein deficiency results in abnormal structure and function of liver thereby leading to fat buildup and fatty livers. Liver does not syntheses plasma albumin leading to oedema. Muscle wasting and anaemia due to the lack of haemoglobin is a common feature owing to the absence of protein. Lack of protein in life could possibly cause mental malfunction raises. FAO (2007) further reported that, young children before age five who are believed to be the most vulnerable to protein deficiency disorders are associated with stunting growth and kwashiorkor. Thus lack of protein leads to delay in growth and in extreme cases failure of growth. The result is marasmus and kwashiorkor among infants and children. Protein deficiency also involves the intestinal mucosa and secretion of digestive enzymes. The outcome is the failure to digest and absorb the food, consequently leading to diarrhoea and loss of fluid (Wu *et al*., 2001).

Protein undernourishment is affected by people who have experienced severe physical trauma that increases protein needs. For example, extensive skin burns, medical condition or psychological challenge that impedes their ability to eat. Adults are also at risk for protein malnutrition (Morens *et al.*, 2003). Brown, (2003) also revealed that in Sierra Leone, the single greatest cause of child mortality is a result of malnutrition thus causing about 46% child death. Protein malnutrition, caused by low intake of protein is also generally in children in underdeveloped countries in Africa, because children need more protein than adults to support the fast growth and development that occurs during childhood (FAO, 2002). According to FAO (2007), About 300 million children worldwide

suffer from growth retardation due to protein malnutrition. Moreover, children with protein malnutrition have a 40% mortality rate, as a result of increased susceptibility to infections. Figure 2.0 shows protein supplies in the world.

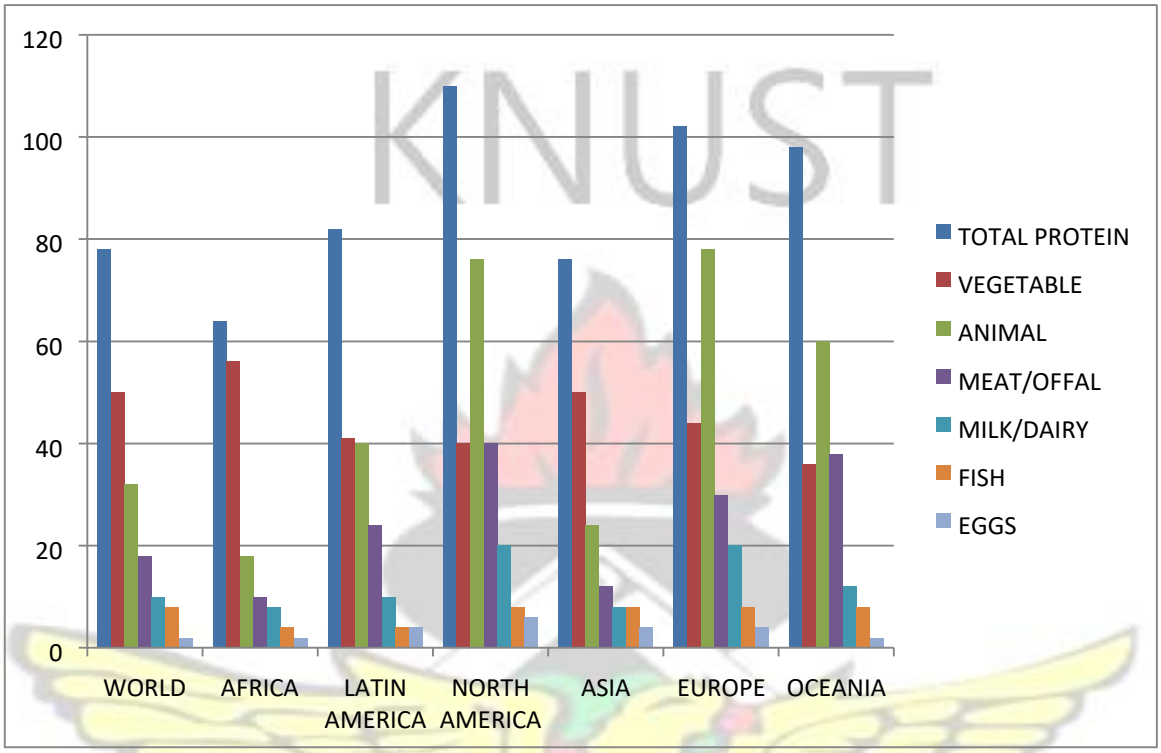

Figure 2.0 Total protein supplies by continent and major food groups

Source: FAO (2012)

Meat according to FAO (2012) is one of the leading sources of animal protein. Fish is an excellent source of protein and the oils contained in fish help protect against heart disease. Small quantities of fish can have a important positive nutritional impact by given essential amino acids, micronutrients and fats that are limited in vegetable-based diets (WHO, 2007). On average, fish offers only about 33 calories per capita per day. It can exceed 150 calories per capita per day in places where there are no different protein food or where fish is the favorite and has been developed and maintained (e.g. Iceland, Japan and several small island States). The dietary input of fish is more significant in terms of animal proteins, as a section of 150g of fish offers about 50–60 percent of the daily protein requirements for an adult (FAO, 2011). Fish proteins can signify a vital component in some

densely populated countries where total protein consumption levels may be low. Many people, especially those in developing countries, rely on fish as part of their daily diet (FAO/WHO, 2011). For them, fish and fishery foods represent a cheaper source of animal protein and also preferred to local and traditional recipes. Fish accounted for 16.6 percent of the world population's intake of animal protein and 6.5 percent of all protein consumed in 2009. Throughout the world, fish provides about 3.0 billion people with almost 20 percent of their average per capita intake of animal protein, and 4.3 billion people with about 15 percent of such protein (FAO, 2012).

In Ghana the average per capita fish consumption is said to be around 20-25kg which is higher than the world average of 13kg. Importantly, as much as sixty percent (60%) of animal protein in the Ghanaian diet country wide is thought to be from fish, which accounts for 22.4% of household food expenditures (FAO, 2002).

Generally fish is a good source of protein and it is low in fat but high in omega-3 fatty acids. Most fish contain 18-35% total solids, 14-20% protein, 0.2-20% fat and 1-1.8% ash (Yada *et al*., 1995). Fakagawu (2014) also reported of fish being the best option in choosing a protein source. This is because of the benefits with regard to growth and development of the body especially in women during gestation, and infants for optimal brain development. On average, fish provides about 33 calories per capita per day (FAO, 2012). For a long time in Ghana, fish has being the favorite and cheapest source of animal protein with about 75% of total annual production being consumed locally. According to Breisinger *et al.* (2011) the importance of fish in the Ghanaian diet cannot be overemphasized as it provides the Ghanaian consumer with about 60% of his or her animal protein needs.

2.2 Amino Acids in Foods

The degree to which dietary proteins can be used for building parts of the human body is resolute primarily by the type and relative amounts of amino acids present in the protein molecule (Lauritzen, 1992). The human body has the ability to interchange and create some of the amino acids. Nevertheless, some amino acids cannot interconvert in the body and as a result, must be supplied by the food we eat. These additional or supplement amino acids are called essential amino acids (Lauritzen, 1992). The more essential amino acids a protein consist of, the higher or better the quality of the protein. Most animal proteins contain all of the essential amino acids in sufficient amounts. Table 2.2 shows some selected protein quality foods.

Table 2.2: Some protein quality foods

High-Quality Protein	Lower-Quality Protein
Eggs	Corn
Milk	Wheat
Meat, fish, poultry	Nut
Soy proteins	Beans, Peas

Source: Keith (2002)

Some proteins tend to be better at being utilized for muscle and tissue-building purposes than others by the virtue of their profile of amino acids. Table 2.3 indicates the essential amino acids in various animal protein sources.

Table 2.3 Essential amino acids in various animal protein sources

	Sources of animal protein			
Amino acids (%)	**Fish**	**Milk**	**Beef**	**Eggs**
Lysine	8.8	8.1	9.3	6.8
Tryptophan	1.0	1.6	1.1	1.9
Histidine	2.0	2.6	3.8	2.2
Phenylalanine	3.9	5.3	4.5	5.4

Leucine	8.4	10.2	8.2	8.4
Isoleucine	6.0	7.2	5.2	7.1
Threonine	4.6	4.4	4.2	5.5
Methionine – Cystine	4.0	4.3	2.9	3.3
Valine	6.0	7.6	5.0	8.1

Source: FAO (2013b)

Sen (2005) also reported a range for free amino acids in taleosts (marine and fresh water fish) and indicated that marine and freshwater fish have 9.1-56.8mg/100g and 34-70.4mg/100g respectively.

2.3 Fish as Protein Source

Fish is a healthy, nutritious and tasty food that is easily digested in the body. Fish is a source of vitamins B (thiamin, riboflavin and vitamin B12. It contains vitamins A and D as well as minerals such as calcium, phosphorous, iron and copper (Salh, 2009). Many people across the globe depend on fish as part of their daily diet. Fish represent a crucial component of food in some densely populated countries where total protein intake levels are low. According to FAO (2013b) it is estimated that around sixty percent (60%) of developing countries depend on fish as source of protein.

Fish protein compares favourably well with other protein rich foods in its amino acid profile (Table 2.3). It contains higher levels of essential lysine and sulphur-containing amino acids (methionine and cysteine) (FAO, 2013). These additional or supplement amino acids are called essential amino acids (Lauritzen, 1992). According to Keith (2002) all proteins contains, some essential and some nonessential amino acids. The more essential amino acids a protein contain, the more valuable it is.

Fish accounts for more than thirty percent (30%) of animal protein supplies in developing countries, while almost eighty percent (80%) of people in the developed world obtain less than twenty percent (20%) of their animal protein from fish. However, with the increased awareness of the health benefits of eating fish and the ensuing rise in fish prices, these figures are rapidly changing. Fisheries are an important part of food security, particularly for many poor people in developing countries (Unit, 2011). For developing countries, fish and fishery products often represent an affordable source of animal protein. Others eat not only because it is cheaper than other animal protein sources, but preferred as part of local and traditional recipes (FAO, 2012).

2.3.1 Other Benefits of Fish

Fish and fishery foods represent an important source of nutrients of fundamental significance for a wide range of healthy diets. Fish is generally low in saturated fats, carbohydrates and cholesterol. With the increasing awareness of high intake levels of saturated fats, consumers are diverting their focus to eating more seafood than red meat (FAO, 2013b). Fish fat or lipids are different from mammalian lipids, because fish lipids consist of long chain fatty acids (14-2 carbon atoms) which are highly unsaturated (4-6 double bonds). The total amount of polyunsaturated fatty acids (PUFA) is slightly higher in lipids from marine fish (88%) than in fresh water 70% (Erickson 1997). Research by Keith (2002) suggests that eating just six ounces (oz) per week of fatty fish, such as salmon or sardines, may be enough to reduce the risk of dying from heart disease by thirty-six percent (36%) and reduce the overall risk of death by seventeen percent (17%). There is clear data on the beneficial effects of fish utilization in connection to coronary heart disease, stroke, age-related muscular deterioration and mental health (Kris-Etherton *et al.*, 2002). Convincing evidence exists now for the significant role fish and fish oils play in

decreasing the risk of developing cardiovascular diseases and improving foetal brain development (Millward, 1999). The FAO in 2013 reported of fish oils containing other essential PUFA which act in similar way as linoleic and arachidonic acids. Linolenic acid has neurological benefits in growing children (Kris-Etherton *et al.*, 2002).

2.3.2 Problems with Fish as Food

Among the food resources of the world, fish and fishery products are very important as sources of food especially in some countries that are unsuitable for livestock production (Olatunde *et al*., 2012). It is known to be one of the cheapest sources of animal protein and other vital nutrients required in human diets (Anihouvi *et al*., 2012). However, Olatunde *et al*. (2012) observed that the quality of harvest is markedly affected by the ease of deterioration and spoilage in fresh fish. After stunning fish, certain changes begin to take place; muscle gradually changes due to chemical, physicochemical, and biological activities. The processes that take place include the activity of the actomysin enzyme during rigor mortis, autolytic degradation, microbial growth and oxidation of fat (Huss 1995). All processes run simultaneously during storage. Okoro *et al*. (2010) also indicated that there are numerous problems confronting the wide field of fisheries and some of which seem to be related to the keeping quality of fish. The onset of rigor mortis owing to loss of the limp elastic texture of the muscle which contracts before becoming hard and stiff are some of the factors. This condition more often than not lasts for a day or more in iced fish, then rigor resolves. Fish deterioration can be detected and assessed with sensory analysis (appearance, texture, flavour, odour and taste), physicochemical and microbial analysis. The flavour of fish changes during the deteriorating process as a result of autolytic degradation. The characteristic sweet, meaty flavour of fish flesh is due to a compound called inosinic acid (Erickson, 1997). The breakdown of inosinic acid during autolysis

results in the loss of this sweet meaty flavour. Hypoxanthine, which is produced from the breakdown of inosinic acid, also contributes to the bitter flavour of spoiled fish. Autolysis also aids indirectly to fish flavours by supplying compounds which bacteria in turn convert to compounds having unpleasant flavour and odour. The colour of muscel may also change during the deteriorating process.

Microbial induced changes occur as a result of bacteria deposited on the skin, gills and in the intestines of live and newly-caught fish. Two pathogenic bacteria that occur as part of the normal microflora of fish and crustaceans are *Clostridium botulinum* type E and non proteolytic type B and F and *Vibrio parahaemolyticus* (Vácha *et al*., 2006). These bacteria according to FAO (2013a) attack the fillet and cause gradual degradation of several of its components (amino acids carbohydrates, nucleotides, and other NPN molecules), producing undesirable volatile compounds such as trimethylamine, volatile sulphur compounds, aldehydes, ketones, esters and hypoxanthine, as well as other low molecular weight compounds. The bacterial load in fish when caught multiply until the fish is consumed. Nevertheless, during handling, fish can probably pick up more bacteria thus from polluted water, careless gutting and dirty boxes. However, if one is cautious when handling, the extent of pollution can be controlled. The growth and activities of bacteria in fish muscle is effected by various environmental changes and the condition of the fish, such as temperature, pH, salinity, water activity (Aw), oxygen and toxic substances (Sofos, 1994). Generally, bacteria grow on a wide range of temperatures. Psychrophilic or psychotropic bacteria grow slowly in chilling and frozen temperatures, but grow faster when the temperature is between 15^{o}C to 20^{o}C (Güngör and Gökoğlu, 2010). The most important bacteria present on fish muscle are *Shewanella putrefaciens, Photobacterium phosphoreum, Pseudomonas spp.* and *Vibrionaceae* (Huss, 1995). Bacteria can also grow

in fish between pH 6 and pH 8 and also show less growth at extremes of pH (Pierard *et al*., 1999).

Oxidation of fat can also affect fish spoilage due to high number of unsaturated lipids (Erickson, 1997). During the period of refrigerated storage, lipids can easily react with other compounds, such as proteins (Mackie, 1993). Lipid spoilage can be noticed by the primary and secondary lipid oxidation products thus interaction between compounds and by lipid hydrolysis (Ocanô-Higuera *et al*., 2011). The primary products are measured as peroxide values (PV) while the secondary products measured as thiobarbituric acid reactive substances (TBARS) and the tertiary products are polymers which can be measured with fluorescence, colorimetric and sensory analysis (Edwards and Ewing, 1972).

According to the FAO (2012), eight percent (8%) of harvested fish food never get to the market; higher values (20 - 40%) of spoilage are recorded in hot humid environment. Moreover, fish spoilage can be as a result of lipid oxidation and hydrolysis leads to the development of rancidity during storage at subzero temperatures. This is because large amounts of polyunsaturated fatty acid moieties are found in fish lipids; this is a major cause of deterioration of frozen fish (FAO, 2013b). Storage facilities which indigenous fish processors use are a problem because of high temperatures and humid climate (Oluwatoyin *et al*., 2010). Eating spoiled fish that have high levels of bacteria can cause illness (Sen, 2005). This has created the awareness on the need to trust and gain knowledge about the quality of fish people consume. It is therefore suggested that critical measures of processing and preservation be taken to enable consumer's attitudes and consumption level of perceived risk and negative consequences be eradicated (Lobb *et al.*, 2007). Post-harvest losses from fish are important especially in developing countries where people use the conventional preservation methods (Tapia de Daza *et al*., 1996).

Brandt (1996) also reported that, fish as a meal is a common and broadly used food, but the bones and smells of fish are considered as unpleasant. The successful application of preservation technology results in the conservation of desirable qualities in stabilized and varietal fish products. Such fish products permit their widespread distribution to meet the needs of people (Tapia de Daza *et al*., 1996). African countries require food processing technologies that will meet the challenges of peculiar food security problems of the continent. Such technologies according to Anihouvi *et al*. (2012) should be low-cost to be affordable by the poor sectors of the community and should be able to address the problems of food spoilage and food borne diseases which are prevalent on the continent (Olapade and Karim, 2011).

2.4 Microorganisms in Meat

Microbial hazards are of concern in the production of animal base foods. Studies associated to microbial contamination have concentrated more on the carcass. However, meat and meat products can be infected with bacteria during processing/manufacturing and packaging. Contamination in sausages from meat, spices, and other non-ingredients, environment, equipment, and handlers during processing affects the microbiological status of the products (Sachindra *et al.*, 2005).

According to Güngör and Gökoğlu (2010) the safety of meat for human consumption has become an essential part of public health debate. Most meat-processing plants have begun to utilize a program called the Hazard Analysis and Critical Control Point (HACCP) system to reduce pathogenic contamination. Food safety aspects of the meat industry were discussed by Hur *et al.* (2008) in sections which consider: the growing awareness of food safety issues during the last two decades; the importance of food inspection services, risk assessment and management in increasing life expectancy among populations in developed

countries. Pavlov and Chukanski (2004) indicated that *Salmonella* infections appear to be increasing in the United State of America (U.S.A) and in other developed nations. *Escherichia coli* O157:H7 is an emerging pathogen, responsible for approximately seventy-three thousand (73,000) cases of infection, two thousand one hundred (2,100) hospitalizations and sixty-one (61) deaths in the U.S.A each year (USDA, 1999). Children and the elderly are more susceptible to developing complications such as hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura (TTP), which may lead to kidney failure. Approximately between two percent (2%) and seven percent (7%) of the infections are associated with such complications. In 2004, there was an important decrease of cases to less than one per 100,000 people (CDC, 2007). Seafood products are vulnerable to food borne pathogenic bacteria such as *Listeria*, *Salmonella*, and *E.coli* (USDA, 1999). Fresh or frozen products can be contaminated by pathogenic bacteria such as *Salmonella*, and coliforms were detected in various processing steps (Pavlov and Chukanski 2004). Therefore, extensive preservation methods were used to process meat to prevent bacterial growth or extend shelf life.

2.5 Fish Processing

Fish like other animal protein sources is a highly perishable product. It has been estimated that in the high ambient temperatures of the tropics, fish spoils within 12-20 hours of harvesting, depending on size and species (Serenius *et al*, 2006). Tapia de Daza *et al*. (1996) indicated that for fish to be stable for future use, it must be free from deterioration in quality from a wide range of reactions that can be physical, chemical, enzymatic or microbiological, or blend of these. Olapade and Karim (2011) stated that fish is generally perceived as having a limited number of uses within the overall pattern of meals, and does not fit into most of our common meal formats. According to Tozer (2001) there are a

number of reasons for processing fish of which some are to supply food that is safe to eat, to minimize loss/waste of valuable food commodity, to meet quality standards and consumer preference, to sell and make profit by adding value and increase convenience for the consumer and also to extend the shelf life so that it could be made available out of season or when it is not possible to catch or purchase fresh food.

Processing fish can be dated back to the last 11,700 years (Zohar *et al*., 2001). Fish processing according to Kondiah (2004) refers to the processes related with fish and fish products between the time fish are harvested, and the time is delivered to the final consumer. These processes may include the following; washing, fining, gutting, heading, filleting, deboning, skinning, cleaning, salting, drying, smoking, grading, canning, marinating, packaging, chilling, freezing, and scaling (Tozer, 2001). These processes can be divided into primary and secondary processing.

Primary processing of fish involves filleting and freezing of fresh fish for onward distribution to fresh fish retail and catering outlets. It also enables fish to be stored or sold for further processing, packaging and distribution. Secondary processing produces

'value-added' products that are chilled, frozen or canned for retail and catering trades (Tozer, 2001). FAO (2012) noted that methods used in fish processing and preservation can be broadly grouped as conventional/traditional or orthodox and modern or advanced processing methods.

2.5.1 Modern Fish Processing

Sen (2005) stated that fish and fish foods are highly-perishable, therefore, they require particularly high demands on hygienic and reliable processing. According to the FAO (2011), fish needs proper handling and preservation if it will be kept for long to ensure good shelf life and keep a desirable quality and nutritional value. Some of the conventional/traditional processing and preservation methods of fish do not ensure good

shelf life quality. Modern technology of processing fish allows for a wide range of products to boost their economic value and allow the fishing industry and exporting countries to reap the full benefits of their aquatic resources (FAO, 2012).

Sen (2005) stated that modern technology has additional benefit with a degree of value addition due to the flexible processing of off-cuts that are produced during the fish filleting process. Moreover, Oduor-Odote and Kazungu (2008) reported that value added processed products creates further employment and high income earnings. Value added products are more important because of modernization that have led to the development of outdoor catering, convenience products and food services requiring fish products that are ready to eat or requiring little preparation before serving. Developed countries have developed value-added fish products like canned fish, minced fish products, fish balls, grilled fish, fish fingers, steaks, fish cakes, cured fish, fish burger, fresh or cooked product, fish loaf and fish sausages which also add cooking convenience and variety to meals (Grunert *et al*., 2004). Sen (2005) also reported of other new processed product like surimi, caviar and sushi, fish pâté, conPro sausages, fish salad and seafood carpaccio. Fish can also be cured and canned (fish in brine), raw cooked and canned (minced fish, flakes) and precooked and canned (e.g. mackerel in tomato sauce, sardine).

2.5.1.1 Canning

Canning uses sterilization techniques that kills microorganisms already present on the fish, stops further microbial contamination, and inactivates degradative enzymes (Metusalach *et al*., 2014). Tapia de Daza *et al*. (1996) stated that canned fish are hermetically sealed in containers and then heated to high temperatures (120^{o}C) to prevent recontamination of the can. Fish for canning is cut into chunks and smaller portions to ensure the entire fillet attains a safe processing temperature. Canned fish products can be stored for several years.

However, sterilization does not kill all microorganisms, and bacterial growth and gas production may occur if the products are kept at very high temperatures. Because the severe thermal conditions of canning cause the breakdown and discolouration of the flesh of many species of fish, only a few types of fish are available as canned products. The most common once are tuna, salmon, herring, sardines and shrimp (Davies and Davies, 2009).

2.5.1.2 Mincing

Minced fish products are examples of value added foods. Minced fish is prepared from the mechanically deboned, washed and stabilized flesh of fish (Sen, 2005). The Minced fish is obtained by passing the eviscerated and beheaded fish or fish waste through a machine which separates the meat from bones. This process allows additional recovery of the meat, in a range of 10 to 20%, of the whole eviscerated fish (Rasekh, 1986). Serenius *et al*. (2006) stated that minced fish is an intermediary product used in the preparation of a variety of ready to eat seafoods such as "*kamaboko*", fish burger, balls, cakes and sausage.

2.5.2 Conventional / Traditional Methods of Processing Fish in Ghana

Fresh fish rapidly deteriorates unless some methods are used to preserve it. The bulk of freshwater and marine fish in Ghana is landed by artisan fishermen. The common conventional or traditional processing methods are salting, smoking, drying, cooking and freezing.

2.5.2.1 Salting

Salting is a old way fishermen used to preserve their fish while at sea and a long way from the market. Salt draws out moisture, drying out the fish thereby preventing an environment where microorganisms can function to cause spoilage (Oluwatoyin *et al*.,

2010). Salting can be classified into two chief techniques: wet salting and dry salting. With wet salting, the fish are immersed in a solution of salt. According to Leistner (2000), wet salting is cheap, since it requires lesser amounts of salt. The theory is to keep the fish for a long time in salt solution. In dry salting, the fish is salted but the fluid, slime and brine are allowed to flow away. Dry salting is done in an old canoe or on mats, leaves, boxes, etc. and it is more susceptible to spoilage and insect attack. Exposure of fish to the air and the presence of salt promote the rate of fat oxidation which gives rise to discolouration and characteristic rancid flavours (Okraku - Offei, 1974).

In Ghana, fish are prepared for salting and drying by gutting, scaling (in the case of tilapia), and washing. The salting is done by placing crude solar salt in the gut cavity and outside of the fish. The fish are arranged in wooden barrels or concrete tubs, with more salt sprinkled on each layer of fish. The ratio of salt to fish has been estimated to be in the range of 1:3 to 1:6 (Nerquaye-Tetteh, 1979). According to Pace *et al.* (1989), salted fish are covered and left for 1-3 days. After salting the fish are removed and spread out to dry in the sun. The salt/brine mixture formed after salting is usually re-used 1-3 times, with more addition of salt. Drying lasts for 3-5 days. Salting is often used in combination with drying and smoking. The presence of sufficient quantities of common salt (sodium chloride) in fish can prevent, or drastically reduce, bacterial action. Oluwatoyin *et al*. (2010) reported that, if the salting process is not carried out properly perhaps due to the use of poor-quality starting materials or addition of insufficient salt, the product could spoil and be lost.

Several factors affect the rates at which salt is taken up and water is replaced in fish. These according Vijayan (1984) include:

(i) Fat content of fish: the higher the fat content, the slower the salt uptake.

(ii) Fish thickness: the thicker the fish, the slower the penetration of salt to the centre.

(iii) Freshness of fish: the fresher the fish, the more slowly salt will be taken up.

(iv) Fish temperature: the higher the temperature, the more rapid the salt uptake.

During subsequent drying the content/concentration of salt in fish has the following effects:

(i) The higher the salt concentration, the greater the replacement of water and, therefore, the less water that remains to be removed during drying;

(ii) The higher the salt concentration, the less water that needs to be removed to produce a satisfactorily preserved product;

(iii) The higher the salt concentration, the more slowly the fish dries;

(iv) Salt tends to absorb moisture from the air and at relative humidity of more than about 75 percent during the drying process or during subsequent storage, fish will not dry further; they may even absorb more moisture.

2.5.2.2 Smoking

Smoking is said to be as old as practice (Nerquaye-tetteh, 1995). Smoke fish means you are drying it out to make sure it is in an environment not favourable for bacteria multiplication. Usually, smoking employs heat to drive off the moisture without cooking the fish (Oluwatoyin *et al*., 2010). In Ghana, smoking is the most widely practiced method preserving fish (Okraku-Offei, 1974). Practically all species of fish available in the country can be smoked and it has been estimated that 70-80 percent of the domestic marine and freshwater harvested fish is consumed in smoked form ((Pace *et al*., 1989). Kagan (1970) reported of two main methods of smoking: **(**a) **Smoking and roasting; (b) hot smoking;** Smoke-roasting refers to any process that has the attributes of both roasting and smoking. This smoking method is sometimes referred to as barbecuing or pit-roasting. It may be done in a smoke-roaster, closed wood-fired oven or barbecue pit.

Hot smoking exposes foods to smoke and heat in a restricted environment. Although we often reheat or cook meats that have been hot smoked, they are safe to eat without further cooking. Hot smoking occurs within the range of 165°F / 74°C to 185°F / 85°C. Within this temperature range, foods are fully cooked, moist, and flavourful. According to Olatunde *et al*. (2012), smoking works best with fillets, but it can be done to steaks or whole fish. Smoking can be a one or two-step process. The one-step process involves taking the fresh cleaned fish and smoking it as- is. The two-step process brines the fish for a time before smoking. This can be done by adding salt for better preservation or flavours for better palatability.

Pace *et al*. (1989) stated that herring, sea bream, and mackerel are the most popular types of fish smoked in Ghana. According to Kagan (1970), the first two types of preserving fish (smoking and roasting) are predominant in the Volta, Greater Accra, Central and Western Regions of Ghana. Pace *et al*. (1989) observed that two methods of fish smoking (hot-smoking and smoke-drying) are generally practiced in Ghana and that smoke-drying is the method most widely practiced. Hot-smoking is usually carried out in the big cities where there is a ready market because hot-smoked fish have a relatively short shelf-life and also cannot be shipped for distances due to their fragile structure. Fish for smoking may or may not be scaled depending on the species. Apart from large species, the guts are usually not removed and the gills are left intact (Nerquaye-Tetteh, 1979). The prepared fish are arranged in layers separated by sticks in the smoking ovens and smoked with materials such as wood chips, coconut husks, etc. A temperature of 50100^{o}C for periods ranging from 3 to 12 hours is usually used in hot-smoking (Leistner, 2000). Approximately 20-40% moisture loss occurs in hot-smoking. Smoke-drying is done at relatively low temperatures for several hours, resulting in a drier product (Okraku-Offei, 1974). This type of smoked fish is the form in which fish is mostly transported from the coasts to remote

areas. Smoke-dried fish has a moisture content of 10-15% and has better keeping qualities than the hot-smoked fish. However, if not appropriately stored and protected, over 50% may be lost through spoilage (Ghaly *et al.*, 2010). According to Vijayan (1984), preserving fish through smoking combines three
effects:

- Preservative value of the smoke: the smoke produced from burning wood contains a large number of compounds, some of which will kill bacteria, e.g. phenols.
- Drying: the fire which produces the smoke also generates heat and this will dry the fish.
- Cooking: if the fish is smoked at a high temperature, the flesh was cooked and this will destroy the enzymes and kill bacteria.

In the traditional system of smoking, natural convection smokers are used in which the fish are hung or laid on openwork trays above fire. The heat from the fire causes a warm column of smoky air to rise and pass over the fish. In other types, a fire is burnt in a pit over which a table carrying the fish is built. Since the sides of the table are open, a considerable proportion of the smoke and heat can escape without passing over the fish (Oluwatoyin *et al*., 2010). A number of designs of smokers have been developed in different parts of the world which utilize locally available materials. Vijayan (1984) [in (Tropical development and research institute)] reported that although these may be very cheap to construct, they tend to suffer from some, or all of the following disadvantages:

(i) They have a high fuel consumption compared to output.

(ii) They have a low capacity.

(iii) They require constant attention.

(iv) They are affected by wind and/or rain.

(v) They are difficult to control and the product is not uniform.

(vi) The materials used in construction are often inflammable.

2.5.2.3 Drying

Vijayan (1984) reported and discussed drying as the removal of water from fish. Normally the term 'drying' implies the removal of water by evaporation but water can be removed by other methods such as the action of salt and the application of pressure. In the traditional method of preserving fish through drying, the actions of the sun and wind are used to effect evaporative drying. In any process of drying, the removal of water requires an input of thermal energy. This thermal energy is obtained from the sun (Islam *et al.*, 2010). During the drying process, considerable shrinkage takes place, as well as other irreversible changes, and dried fish will not reconstitute to their original condition.

With air drying, water is removed from the surface of the fish, and water moves from the deeper layers to the surface. Drying takes place in two distinct phases. In the first phase, whilst the surface of the fish is wet, the rate of drying depends on the condition (velocity, relative humidity etc.) of the air around the fish. If the surrounding air conditions remain constant, the rate of drying will remain constant; this phase is called the 'constant rate period'. During this period the rate of drying depends on the speed at which moisture can be carried away from the surface of the fish. Several factors according to Leistner (2000) influence the rate of drying:

(i) Relative humidity (RH) of the air: if the air is fully saturated with water vapour (relative humidity 100 percent), it cannot carry any more water and no drying of the fish will occur. If the RH is less than 100 percent, the air has the ability to absorb water and drying will proceed; the lower the RH, the greater the ability to absorb water and the faster the rate of drying.

(ii) Air velocity: the greater the speed of the air over the fish, the greater the drying rate. The air around fish can be considered as three layers: a stationary layer close to the fish, a slowly moving layer outside this and an outer turbulent layer. The stationary layer of air next to the fish is saturated with moisture that passes into the slowly moving layer. The higher the air speed in the outer layer, the thinner the slow moving layer. This allows more rapid movement of water away from the fish.

(iii) Air temperature: the evaporation of water produces a cooling effect. At the beginning of drying, the temperature of the fish is reduced below ambient; after a short while it reaches a steady value. At this steady value, the heat energy required for evaporation is balanced by the heat supplied by the surrounding air. The degree of cooling is related to the wet bulb depression of a hygrometer and reflects the ability of the air to hold water. Warm air can provide more heat energy and, provided that the air speed and relative humidity will allow a high rate of water movement, the rate of drying will be increased.

(iv) Surface area of the fish: the larger the surface area, the faster the rate of drying. If a fish is split, the surface area increases relative to the weight/thickness; the rate of drying will, therefore, be faster.

Once all the surface moisture has been carried away, the second phase of drying begins and this depends on the rate at which moisture can be brought to the surface of the fish. As the concentration of moisture in the fish falls, the rate of movement of moisture to the surface is reduced and the drying rate becomes slower; this phase is called the 'falling rate period'. Once the free surface moisture has been removed, the rate of drying depends on the movement of moisture to the surface of the fish. Several factors influence the rate of drying:

(i) The nature of the fish: a high fat content in the fish retards the rate of drying.

(ii) The thickness of the fish: the thicker the fish, the further the water in the middle layers has to travel to reach the surface. Leistner (2000) observed that very small and thin fish can be dried straight away in the sun if they are brought in early enough in the morning and the sun is shining.

(iii) Temperature of the fish: diffusion of water from the deeper layers to the surface is greater at higher temperatures.

(iv) The water content: as the water content falls, the rate of movement to the surface layers is reduced (Nerquaye-Tetteh, 1979).

Okraku-Offei (1974) observed that by far, sun-drying is the cheapest and simplest of the methods used in Ghana for preserving fish. However, this is mostly used in combination with salting and/or fermentation for effective preservation of the product. Ten to fifteen percent of fish landed in Ghana are preserved by straight sun drying. Anchovies, sardines, trigger, tilapia, and moonfish are examples of fish that are sun-dried. Usually the fish are washed and spread on the ground (beach sand or fine gravel) or on mats and allowed to dry for 3-5 days (Nerquaye-Tetteh, 1979).

2.5.2.4 Freezing

Vácha *et al*. (2006) stated that of the many processing methods used to preserve fish; only freezing can maintain the flavour and quality of fresh fish. Freezing greatly reduces or halts the biochemical reactions in fish flesh. The three steps for freezing fish include immediate cooling and holding, rapid freezing, and cold storage. If fish is frozen improperly, structural integrity may be compromised because of enzymatic degradation, texture changes, and dehydration.

In immediate cooling there is rapid cooling and holding of fish at temperatures between 2°C and −2°C (36°F and 28°F) immediately after the fish has been harvested (Burgaard

and Jorgensen, 2011).With rapid freezing, the key is rapid reduction of temperature to between −2°C and −7°C (28°F and 20°F). This temperature range represents the zone of maximum ice crystal formation in the cells of the flesh. If water in the cells freezes quickly, then the ice crystals will remain small and cause minimal damage to the cells (Olatunde *et al*., 2012). Cold storage of fish is where fish is frozen and stored at a constant temperature of −23°C (−10°F) or below in order to maintain a long shelf life and ensure quality (Sanchez and Swaminathan, 2005). A large portion of fresh fish is water. Because the water in fish contains many dissolved substances, it does not uniformly freeze at the freezing point of pure water. Instead, the free water in fish freezes over a wide range, beginning at approximately −2°C (28°F). The amount of remaining free water decreases until the product reaches a temperature of approximately −40°C (−40°F). Fish held below this temperature is packaged so as not to allow water loss through sublimation. Unfortunately, there are relatively few commercial freezers capable of storing fish at -40°C because of the tremendous variation in energy costs. Fish are therefore normally stored at −18 to −29°C (0 to −20 °F), resulting in a variable shelf life ranging from a few weeks to almost one year (Davies and Davies, 2009).

2.6 Modern Methods of Processing Meat

When viewing meat products of various size, shape and colour in butcher shops or meat sections of supermarkets, there is great variety of products with different taste characteristics. Several different meat products exist in both developed and developing countries with individual product name and taste characteristics. Based on the processing technologies used and taking into account the treatment of raw materials and the individual processing steps, it is possible to categorize processed meat products in six broad groups (FAO, 2013a) as shown in Fig. 2.1

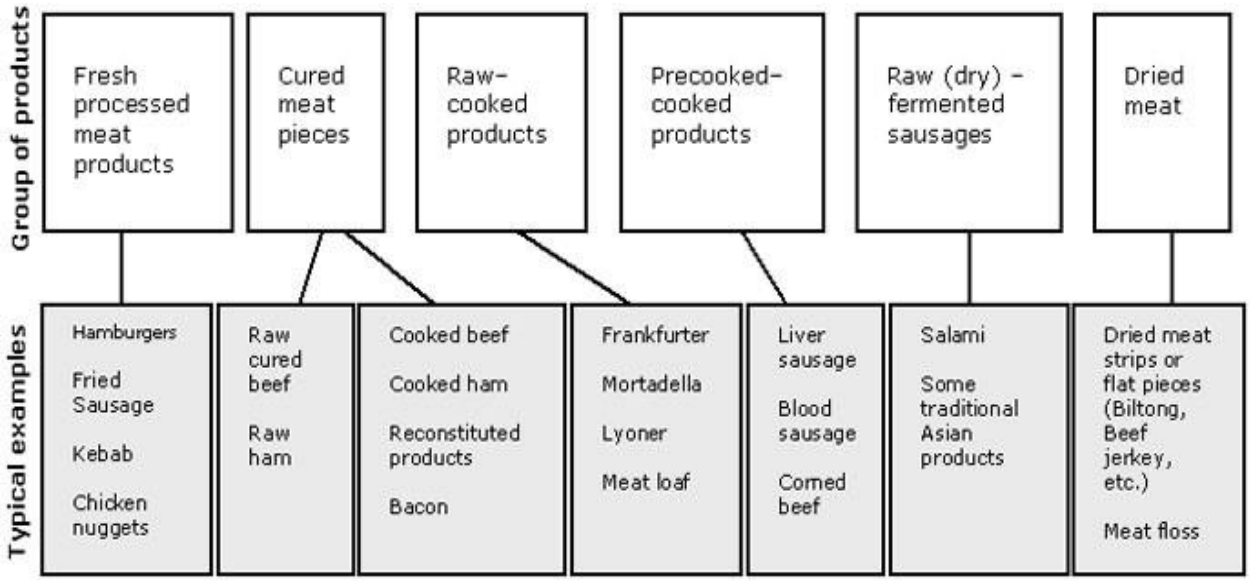

Figure 2.1 Meat products grouped according to the processing technology applied

Source: (FAO, 2013a).

2.7 Sausages and their Classification

Okanovic *et al*. (2013) stated that sausages may be classified in many number of ways, for instance by the type of meat and other ingredients they contain, or by their consistency or method used in their manufacture. The most popular classification is probably by type of preparation; but even this is subject to regional differences of opinion. For instance, in the English-speaking countries, distinction is made between fresh sausages, cooked sausages and dry sausages (Dinstel, 2013). Others according to Bates (1985) have several basic categories of sausages depending on the method used in manufacture namely, fresh sausage, cooked sausage, cooked and smoked sausage, uncooked and smoked sausage, dry sausage and specialty meats.

Classification based on the type of meat and ingredients are based mainly on meat of only one species; examples include fish, chicken, beef and pork sausages. But it is very common to use two or three types of red meat and poultry ingredients in many types of sausage formulations (Yilmaz *et al.*, 2002).

2.7.1 Fish Sausages

Sausage formulation is an art and more than 200 different varieties of sausage are made worldwide, varying by regional tastes and ingredient availability (Sen, 2005). Anihouvi *et al*. (2012) reports that, early sausage makers have found that a wide range of raw ingredients could be used. The great variety of sausages makes it possible to serve many different products, each having its own characteristic appeal and flavour. Sausage products take little time to prepare, with some being ready to serve and others needing only to be warmed before serving, commonly for breakfast, lunch, dinner or snacks (Pearson and Gillett, 1999).

Raju *et al*. (2003) indicated that fish sausage like other meat sausages is comminuted seasoned fish products that may also be cured, smoked, shaped, and heat treated. The degree of comminuting varies widely; some are very coarsely comminuted with individual particles easily visible like smoked fish sausage. Others are finely divided that individual particles are not easily visible. The sausage mix usually looks like a viscous mass with characteristics of an emulsion. They are referred to as batter-type sausages because in the true sense they are not emulsions (e.g. fish frankfurters) (USDA, FSIS and AFDO, 1999).

Fish sausage is produced by using the fish flesh which is mixed with additives, stuffed into suitable casings and heat processed. FAO (2011) reported that, fish (all species) can be used in sausage preparations, but Tanikawa (1971) had reported that, although a wide range

of fish species can be used, dark muscled fish such as mackerel and tuna are not recommended as raw materials since they produce dark blackish red spots after processing. With classification based on type of cooking, there are several basic categories of fish sausages, namely, fresh sausage, cooked sausage, cooked and smoked sausage, uncooked and smoked sausage, dry sausage and specialty meats (Bates, 1985).

2.8 Ions (Minerals) in Meat

Minerals are the nutrients that exist in the body, and are as essential as our need for oxygen to sustain life. Minerals are also found in organic and inorganic combinations in food (McDowell *et al*., 1986). The human body weight is composed of only 5% of mineral matter, vital to all mental and physical processes and for total well being. Minerals are most important factors in maintaining all physiological processes. They are constituents of teeth, bones, tissues, blood, muscle, and nerve cells (Hays and Swenson, 1985). Minerals function along with vitamins as essential components in enzymes and coenzymes. If an enzyme is lacking the necessary mineral, it cannot function properly no matter the vitamin available (Meeker *et al.*, 2010). The importance of mineral elements in human, animal and plant nutrition has been well recognized (Mcdowell *et al*., 1986).

Minerals are classified into two categories: major and minor. This classification is determined by the amount of the mineral needed by the body, not by how essential it is to good health. If a mineral is required at a level greater than 100 mg per day, it is considered to be a major mineral (Simsek and Aykut, 2007).

Major minerals include Calcium, Phosphorus, Potassium, Sulfur, Sodium, Chlorine, and Magnesium while minor (also known as “trace”) minerals include Zinc, Iron, Manganese, Copper, Boron, Silicon, Molybdenum, Vanadium, Chromium, Selenium and Iodine (Hays and Swenson, 1985).

Mineral elements are separate entities from the other essential nutrients like proteins, fats, carbohydrates, and vitamins. Human nutrition had demonstrated the need for minerals in a diet (Hegsted *et al*., 1976). Simple or conditioned deficiencies of mineral elements have profound effects on metabolism and tissue structure (Simsek and Aykut, 2007). Figure 2.2 shows average total body content of major minerals.

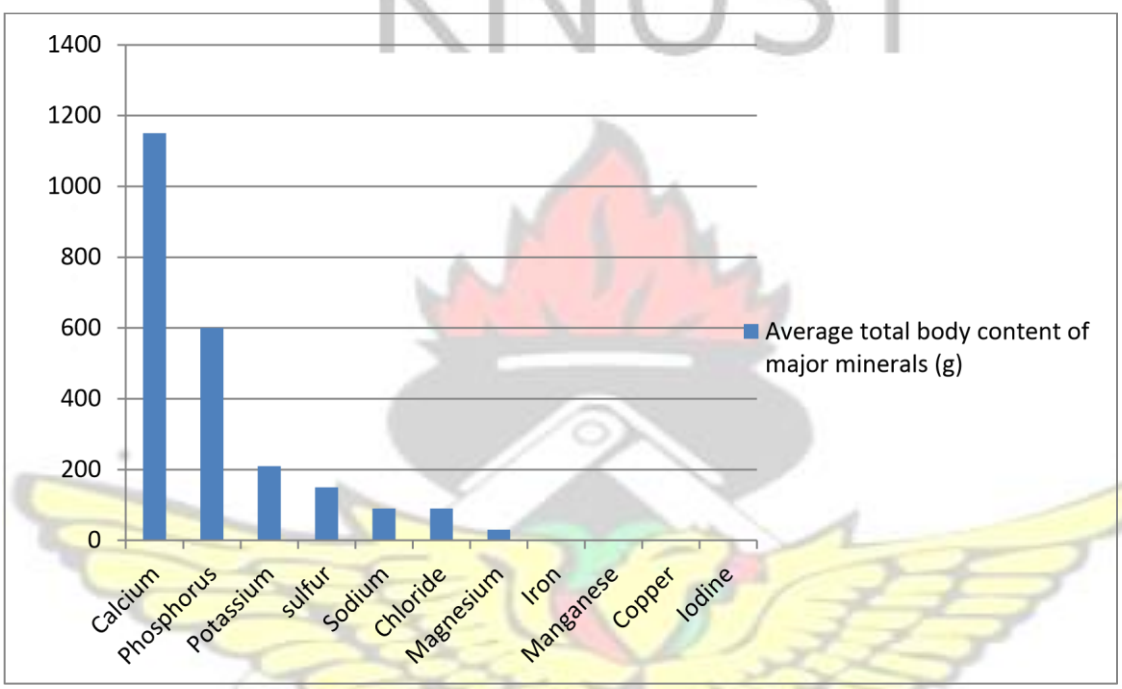

Figure 2.2 Average Total Body Content of Major Minerals (g) Source: Hays and Swenson (1985).

Mineral elements play important roles in health and disease states of humans and domestic animals. For example, deficiency in iron causes anaemia and goiter. Iodine deficiency is reported to be problems of public health importance in some countries (Deosthale and Belavady, 1978). Trace elements like zinc and selenium are significant in the human diet especially to people with HIV (ARN, 1996). Selenium is an antioxidant that increases immune function. Zinc is usually taken to stimulate the immune system, it has been reported to affect immune system function and lower calcium levels in HIV – positive men (O' Connor, 1995).

Infants deserve extra concern because they need adequate micronutrients to maintain normal growth and development (Lopez *et al.*, 2002). The micronutrient deficiencies of greatest public health significance are iron deficiency, causing varying degrees of impairment in cognitive performance, lowered work capacity, lowered immunity to infections, pregnancy complications e.g. babies with low birth weight, poor learning capacity and reduced psychomotor skills (Batra and Seth, 2002). Chakravarty and Ghosh (2000) reported that any severe case of anaemia is a direct cause of maternal and child mortality.

The functions of minerals in humans are inter-related. Examples are the definite relationship of calcium and phosphorus in the formation of bones and teeth and as the major structural elements of the skeletal tissue. Hypocalcaemia may cause weakness of the heart similar to that caused by hyperkalaemia. High level of potassium increases the requirement for sodium and vice versa (Merck, 1986). Chakravarty and Ghosh (2000) reported that, potassium deficiency can lead to an increase in the basic amino acid concentration of the tissue fluids and some increase in cellular sodium levels as a means of maintaining cation-anion balance. Simsek and Aykut (2007) further reported of the influence of potassium on the contraction of smooth, skeletal, and cardiac muscles and its effect on muscular irritability that, like that of sodium, tends to antagonize the effect of calcium ion.

Under conditions of salt restriction, calcium appears highly important in helping to maintain the potassium content of tissue (Hays and Swenson, 1985). The plasma phosphorus level is inversely related to the blood calcium level. There are also interrelationships of iron, copper and cobalt (in vitamin B12) in haemoglobin synthesis and red blood cell formation (Hays and Swenson, 1985).

2.9 Water Holding Capacity (WHC) of Meat

The water holding capacity of meat products is a very important quality attribute which has an influence on product yield, which in turn has economic implications as well as eating quality (Van Laack, 1999). A number of factors such as pre-and post-mortem factors influence the water holding capacity (WHC) of meat. It is clear that early postmortem events including rate and extent of pH decline, proteolysis and even protein oxidation are key in influencing the ability of meat to retain moisture (Cheng and Sun 2008). As rigor progresses after slaughter, the space for water to be held in the myofibrils is reduced and fluid can be forced into the extra myofibrillar spaces where it is more easily lost as drip (Lonergan and Lonergan, 2005). A considerable amount of water is exuded from fish muscle tissue after death due to muscle contraction during the rigor process (Sen, 2005).

Other relevant factors like growth and development of meat animals/species, genotype and animal diet are important due to their direct influence on muscle characteristics (Cheng and Sun, 2008). Much of the water in the muscle is entrapped in structures of the cell, including the intra- and extra myofibrillar spaces; therefore, key changes in the intracellular architecture of the cell influence the ability of muscle cells to retain water. In the immediate pre-slaughter period, stresses on the animal such as fasting, and different stunning methods are likely to influence meat WHC. In the post-slaughter period chilling, ageing, injecting non-meat ingredients, as well as tumbling have important influences on WHC. Furthermore, cooking and cooling procedures for the final meat products can also affect the WHC of the product, in particular, the cooking and cooling methods, the heating and the cooling rate, the cooking temperature, and endpoint temperature (Cheng and Sun, 2008).

2.10 Inferences from Literature

Based on the literature reviewed, the following inferences can be made: protein is required in the diets of humans for the repair and maintenance of body tissues. Also proteins especially those of animal sources are said to be of better quality compared to plant sources. However, in developing countries such as Ghana, insufficient quantities of animal proteins are attained since the prices of most meat and animal products may be described as expensive for most people living in third world countries.

Fish, one of the most abundant and cheaper sources of animal protein in developing countries is still not meeting expected consumption rate due mainly to the fact that fish is highly perishable especially in hot humid conditions. Moreover, very few fish products exist on the Ghanaian market. These fish processed products are mainly of primary origin ("Kobi and Momone"). Therefore, for people selling fish products to maximize their profits and improve shelf-life of their products, value addition is cardinal since there are inadequate storage facilities and those few outlets with storage facility are not in good condition. Thus most local people who trade in fish and other fish products in Ghana have resorted to salting, smoking, drying, cooking and in some cases freezing. However, in the developed world, a variety of processed fish products such as, minced fish, fish balls, fish burgers, fish loaf, fish sausages, etc. exist and this may also account for their high intake of fish and fish products. The need to research to produce a variety of fish products with longer shelf-life in countries like Ghana can therefore not be overemphasized.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Location and Design

The experiment was carried out at the Meat Science and Processing Unit, Department of Animal Science, KNUST. Four treatments: (T1) = Pork frankfurter without corn starch; (+VE PFS) served as the positive control; (T2) = Mackerel frankfurter (MFS); (T3) = Catfish frankfurter (CFS) and (T4) = Pork frankfurter with corn starch (-VE PFS), negative control.

Pork frankfurter was produced to enable consumers differentiate between the sausage types. The experimental design was Complete Randomized Design (CRD) with three replicates and 2×4 factorial arrangement for the determination of sensory and proximate components.

3.2 Source of Raw Materials and Initial Preparation

Frozen Mackerel, fresh Catfish and boneless Pork thigh were respectively obtained from the Felibat cold store, Akate Farms Company Limited and Kumasi Abattoir Company Limited, all in Kumasi, Ghana. Other non - meat ingredients used in the formulation were purchased from the Ayeduase market except ice, which was produced from the Department of Animal Science, KNUST. Fresh catfish were stunned and gutted after beheading (Fig 3.0). Frozen mackerel were beheaded and gut. The beheaded and eviscerated portions were kept under refrigerated temperatures overnight. Using a pair of pliers, the skin of the catfish was removed (Fig 3.1). A knife was then carefully positioned at the tail fin and cut laterally along the mid rib to obtain filleted boneless fish muscles (Figures 3.2 and 3.3).

Plate 3.0: Evisceration and gutting of catfish

Plate 3.1: Skinning catfish

Plate 3.2: Deboning catfish

Plate 3.3: Deboning mackerel

Boneless mackerel and catfish were weighed separately as well as the entrails. Entrails were quickly disposed in order to prevent bacterial accumulation. Filleted muscle were frozen and later minced.

3.3 Fish Sausage Preparation

3.3.1 Mincing

Filleted frozen catfish, mackerel, lean pork and pork fat were minced separately using a Mincer (MA®Superwolf, Germany) with a grinding sieve of 5mm and 3mm, respectively. Different sieve diameters were used because the meat ingredients have different muscle fibre, and also to prevent mashing.

3.3.2 Sausage Formulation

Table 3.0 shows the formulation used in preparing the sausages and Fig. 3.4 is a flow chart of the production processes. Each treatment was chopped in a bowl cutter. Dry ingredients were added, then ice and finally fat (for the pork frankfurter).

Table 3.0 Ingredient used in sausage formulation

Ingredient (%)	Frankfurter- type			
	T1=PFS (+VE)	T2 = MFS	T3=CFS	T4=PFS (-VE)
Minced mackerel	0.00	70.40	0.00	0.00
Minced catfish	0.00	0.00	70.40	0.00
Minced pork	67.40	0.00	0.00	65.40
Minced pork fat	5.00	0.00	0.00	5.00
Curing salt*	1.2	1.2	1.2	1.2
Phosphate **	0.7	0.7	0.7	0.7
Corn starch	0.00	2.00	2.00	2.00
Mixed spices***	0.70	0.70	0.70	0.70
Ice flakes	25.00	25.00	25.00	25.00
Total	100.00	100.00	100.00	100.00

PFS (+VE) = Pork Frankfurter without corn starch, MFS= Mackerel Frankfurter, CFS= Catfish Frankfurter, and PFS (-VE) = Pork Frankfurter with corn starch
*Curing salt was added in the form of sodium nitrite (4g of nitrite to 10Kg common salt)
**Phosphate was added in the form of disodium phosphate (Na_2HPO_4)
*** Mixed spice = chilli pepper (0.3%), black pepper (0.125%), garlic (0.125%) and nutmeg (0.15%)

Each mixture was finely chopped and filled using a manually operated stuffer into 26mm diameter hog casings, and tied off in desired sausage lengths. The sausages were hot smoked for 45 m and scalded in 70°C water for 30 m. A meat thermometer was used to monitor the core temperature of the sausages in order to prevent over cooking. The scalded sausages were allowed to cool under running tap water, packaged and labeled for storage in a freezer for quality analysis and for further studies.

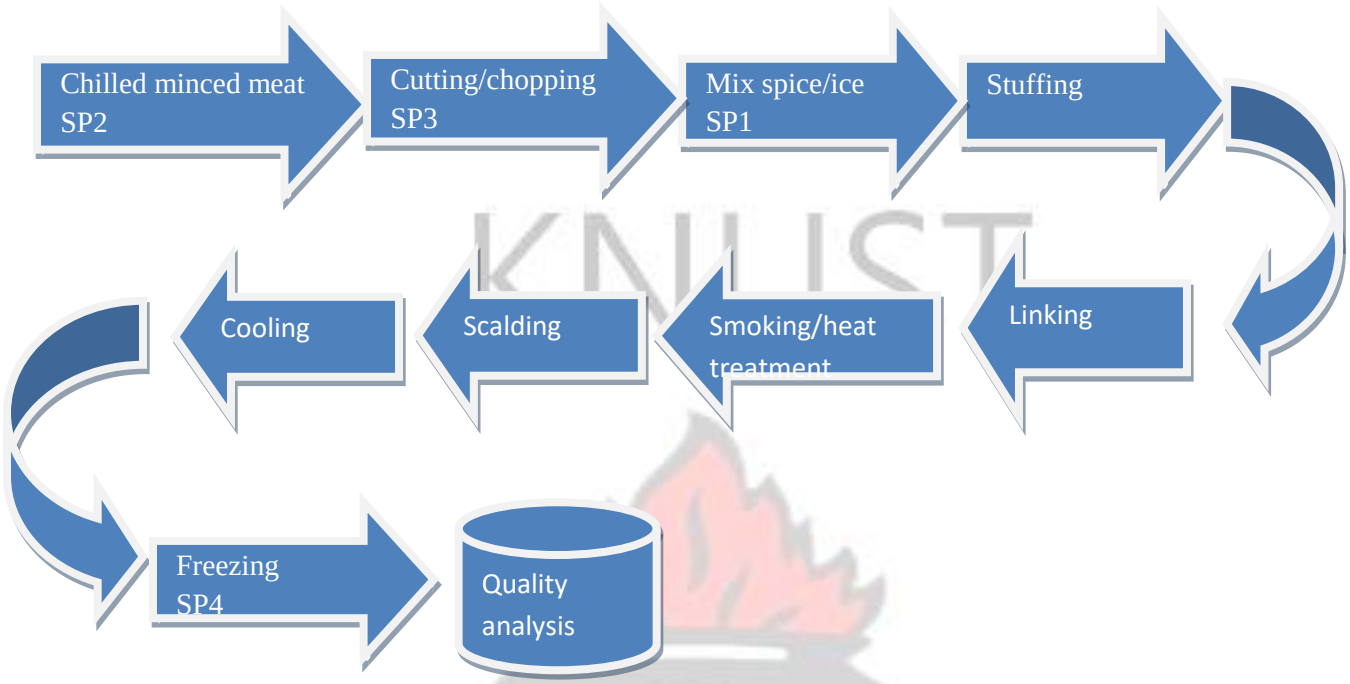

Figure 3.4 Flow Chart for sausage production

3.4. Parameters Measured

3.4.1 Physicochemical characteristics

Proximate analysis (moisture, ash, fat, crude protein and carbohydrates) was carried out at the Soil Chemistry Laboratory of the Crop Science Department, KNUST, using AOAC (1990) procedures (Appendix 7.1). Ash was determined according to AOAC method 923.03.

Acidity (pH) was determined at the Meat Science and Processing Unit using the procedure described by Bates and Vijh (1973) (See Appendix 7.2). Water holding capacity was determined as described by (Suzukie et al., 1991) (Appendix 7.3).

3.4.2 Amino acids and Ions determination

Amino acid profile was determined using High Performance Liquid Chromatography

(HPLC) (Appendix 7.4). Mg^{2+}, $Ca2^{+}$, K^{+}, NH_4^{+} and Na contents were determined by Cadmium.mtw and ASUP5 – 100 marvin.mtw used for anions (Fl^{-}, Cl^{-}, $N0_3^{-}$, $S0_4^{2-}$ and $P0_4^{-}$). Cation and anions in meats and their corresponding sausages were determined by Cadmium.mtw and ASUP5-100 marvin.mtw respectively (Appendix 7.5).

3.4.3 Microbial safety [Total Viable Count (TVC) and Isolation of *Escherichia coli*]

Total microbial load and presence of *E. coli* were determined weekly as described by (ICMSF 2002) for 6 weeks of frozen storage (Appendix 7.7).

3.3.4 Sensory Attributes

Samples were evaluated in week one and six after processing. Untrained consumer panelists (n=45) including Lecturers, students, and regular sausage consumers recruited from Kwame Nkrumah University of Science and Technology evaluated the frankfurters based on how they liked product appearance, juiciness, texture, flavour and after taste characteristics. All participants were regular consumers of frankfurters and other processed meats. The panelists evaluated the frankfurters using a 9-point Hedonic scale (9 = like extremely, 5= neither like nor dislike and 1 = dislike extremely). The products were sliced to approximately equal lengths of 2 cm, coded with 3-digit random numbers and oven warmed at 180°C for 2 minutes before serving. In order to control individual differences between panelists, the order of serving samples was randomized and counter balanced so that all treatments occurred equally. Biscuit was offered alongside test samples for the consumer panelists to eat between testing samples in order to neutralize the sensory profile of each test sausages. The taste evaluation took place under conditions (Mackie *et al*., 1991) that ensured independence throughout the entire duration. The room was well illuminated with white fluorescent lights and there was no noise or awful odour that could possibly distract the attention of panelists.

3.4 Statistical Analysis

Data obtained for pH, water holding capacity, amino acids and ions were subjected to one-way analysis of variance (ANOVA) while sensory and proximate parameters were subjected to 2 x 4 factorial analysis. pH, water holding capacity, amino acids and ions were analysed using while sensory and proximate parameters used the GLM Procedure (SAS System 2012) significant differences between means were separated at 5% by Least significant difference test.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Acidity (pH) and Water Holding Capacity (WHC)

Low pH and accelerated pH decline are related to the development of low water-holding capacity and consequently high purge due to denaturation of many proteins (Lawrie and Ledward, 2006) especially those concerned with muscle functionality.

Treatment T1 and T4 recorded higher pH ($p<0.05$) in both raw and cooked sausages while the observed pH in T2 and T3 were similar in both raw and cooked frankfurters (Table 4.1). The high pH observed in T1 and T4 could be due to prolonged stress prior to slaughtering the pigs, and consequently resulting in Dry Firm and Dark (DFD) pork. Barbut (1993) reported that stress, accelerated post-mortem metabolism and other biochemical changes can affect pork muscle quality postmortem. The result of which is high pH and increased water holding capacity. Van de Perre *et al*. (2010) determined the influence of stunning on pH of pork and observed that some risk factors like the noise level produced during unloading, the rate of panting and the use of an electric prod can affect the pH and temperature of meat. According to Serenius *et al*. (2006) lowering the temperature of carcass decreases metabolic processes and reduces the rate of pH decline. Meats with pH below 5.4 have the tendency of increasing firmness in processed products (Lonergan and Lonergan, 2005). Thus T1 and T4 were expected to be relatively less firm compared to T2 and T3 because of their relatively low pH (Leistner and Roedel, 1995). Lawrie and Ledward (2006) stated that high pH improves juiciness of products. The high acidity observed in T2 and T3 could favour decreased microbial attack (Leistner and Roedel, 1995) because reduced pH media create unfavourable conditions for microbial activities and thereby prolonging the shelf-life of such product.

Treatment one (T1) recorded the highest WHC while T2 had the least in both emulsion and sausage (Table 4.0). The observed differences in WHC for both emulsion and cooked products were significantly ($p<0.05$) different. T2 which recorded the highest acidity was observed to hold less water in both emulsion and sausage. This supports the report that a lower pH results in reduced WHC of final products (FAO, 2007). T3 confirms this report in the both sausage and in the emulsion. Water holding capacity measured as drip loss has high importance in meat production because of its' financial implications. In general it can be said that meat with a low WHC has an unattractive appearance (Lawrie and Ledward, 2006; Santos *et al*. 1994). The findings of this study support Lawrie and Ledward (2006) because T2 scored low for appearance during sensory evaluation (Table 4.3). Also, a high WHC in emulsion and sausage could be attributed to much more stable meat protein matrix formed which led to smaller release of water and fat, thus improving binding properties (Fennema, 1990).

According to Hamm (1985), there are many factors that influence the water holding capacity of muscle tissue. These are internal or external factors. Species, age, size, muscle type, amount of intra muscular fat and muscle tissue condition post mortem forms the internal factors. External factors include feeding patterns, season and location of catching and handling post slaughter. According to Shenouda, (1980), reduced WHC in fish frankfurter (catfish and mackerel) can be primarily due to denaturation/aggregation of actin and myosin (thin filament and thick filament respectively) that are responsible for toughness and tenderness depending on once bridge between them. Actin and myosin which are the main contractile proteins are responsible for functional properties (Shenouda, 1980). The WHC is affected by the changes that take place in muscle tissue

post mortem. Changes in chemical composition during processing, like salting could also have influenced WHC (Fennema, 1990).

In general, pH is commonly known to be one of the most important factors to affect the WHC of a product (Hamm 1985; Nott *et al.*, 1999).

Table 4.0 Acidity (pH), Water Holding Capacity and Cooking Yield Parameter

Frankfurter- type	SEM T1	T2	T3	T4	
pH (Emulsion)	5.60^{a}	5.35^{b}	5.38^{b}	5.58^{a}	0.01666
pH (Sausage)	5.78^{a}	5.41^{c}	5.44^{c}	5.63^{b}	0.00726
WHC (Emulsion %)	24.60^{a}	7.90^{c}	22.41^{a}	19.07^{b}	1.01959
WHC (Sausage %)	16.89^{a}	4.32^{c}	16.66^{a}	13.78^{b}	1.00024
Cooking Yield (%)	68.04^{d}	79.07^{b}	83.03^{b}	91.55^{a}	1.44705

T1= pork frankfurter without corn starch, T2= mackerel frankfurter, T3 = catfish frankfurter and T4 = pork frankfurter with corn starch

4.2 Cooking Yield

Treatment four T4 recorded the highest ($p<0.05$) cooking yield while T1 recorded the least. The high cooking yield (91.55%) observed in T4 could be as a result of the corn starch added, which increased its moisture retention capacity (Fennema, 1990). Corn starch contains carbohydrates that contributed to water absorption during emulsification. Thus, T4 absorbed and retained much moisture during cooking compared to T1 which did not contain corn starch. Brown and Zayas (1990) made similar observations when corn flour was used as meat extenders. There were no differences ($p>0.05$) between T2 and T3 in terms of cooking yield; and both T2 and T3 yielded higher ($p<0.05$) than the positive control (T1). The observed differences could be due to the meat type used. According to Peng *et al.* (2009) processing yield could be affected by type of meat used. This could be attributed to higher protein content of fish than pork which probably improved functionality of the products thereby decreasing cooked losses (Fakagawu, 2014). Thus lower cooking yield in T1 can be attributed to the absence of corn starch (extender).

FAO (2001) reported that the flesh of mackerel contains about 60-74% water. This could be one of the reasons accounting for poor WHC of mackerel affecting the cooking yield of mackerel sausage.

4. 3 Cost of Production

A simple analysis of the ingredients used in product formulations (Table 4.1) revealed that the costs (Gh¢) per kg were respectively 6.70, 6.10, 7.42 and 6.52 for T1, T2, T3 and T4.

Table 4.1 Cost of ingredients used in sausage production

Ingredient (GH¢)	**Frankfurter- type**			
	T1	T2	T3	T4
Pork fat	1.8	0	0	1.8
Minced meat	28.39	24.20	32.07	27.55
Corn starch	0	1.32	1.32	1.32
Total cost	30.19	25.52	33.39	30.67
Cost of Production (Gh¢)/kg	9.85	7.72	8.94	7.12

NB: Cost of production is less spice, phosphate, curing salt, casings, labour, machinery and water
T1= pork frankfurter without corn starch, T2= mackerel frankfurter, T3 = catfish frankfurter and T4 = pork frankfurter with corn starch

4.4. Proximate Composition

Table 4.2 shows the proximate composition obtained from the various treatments during this study. Significant differences ($p<0.05$) were observed for moisture, protein, fat and ash contents among treatments. T3 (catfish frankfurter) recorded the highest values for all proximate components analysed with exception for fat. It contained 18.86% protein and 10.45% fat which support the findings of (Okanović, *et al*. 2013).

Table 4.2 Proximate composition of sausage used (As - In) during storage Source

Moisture	Protein	Fat	Carbohydrate	Ash Frankfurter- type	
Week 1	58.14[b]	17.29[a]	17.72[a]	2.16[b]	5.10[a]
Week 6	62.77[a]	11.65[b]	18.25[a]	2.76[a]	4.61[b]
SEM	0.39	0.22	0.21	0.14	0.14
F.pr.	<.0001	<.0001	0.0888	0.0085	0.0273
Interaction					
T1	60.02[b]	14.26[b]	19.27[b]	1.89[c]	4.37[b]
T2	55.98[c]	13.29[c]	23.30[a]	2.24b[c]	5.56[a]
T3	64.87[a]	15.69[a]	10.66[c]	3.21[a]	5.92[a]
T4	60.95[b]	14.64[b]	18.69[b]	2.51[b]	3.57[c]
SEM	0.55	0.31	0.29	0.20	0.20
F.pr.	<.0001	0.0005	<.0001	0.0022	<.0001
Storage					
T1 x Week 1	57.24	16.85	19.06	1.18	4.74
T1 x Week 6	62.79	11.67	19.48	2.59	4.00
T2 x Week 1	53.48	16.13	23.21	2.10	5.88
T2 x Week 6	58.48	10.44	23.39	2.38	5.23
T3 x Week 1	62.86	18.86	10.45	2.90	6.04
T3 x Week 6	66.89	12.517	10.87	3.51	5.80
T4 x Week 1	59.00	17.30	18.15	2.44	3.72
T4 x Week 6	62.91	11.98	19.23	2.58	3.42
SEM	0.78	0.44	0.41	0.29	0.28
F.pr.	0.6824	0.5564	0.7313	0.1544	0.7624

Superscript [abcd] across rows are significantly different ($p<0.05$)
T1= pork frankfurter without corn starch, T2= mackerel frankfurter, T3 = catfish frankfurter and T4 = pork frankfurter with corn starch

Okanović *et al.,* (2013) indicated that fish sausage has higher protein (17.32%) and less fat (21.06%) content than sausages made from other farm animals' meat. FAO (2001) also reported lesser fat range in mackerel (1.0% to 23.5%) and catfish (2.1 to 3.8%) as well as with protein (16% to 20%) and (17% to 19%) in mackerel and catfish respectively.

However, protein and fat results from this study (T3) were higher, and the moisture content was lower than that reported by (IoM, 2002). IoM (2002), had earlier published that sausages made from catfish contained 74.5% water, 3.16% fat and 13.73% protein. T1 (pork sausage without corn starch) and T4 (pork sausage with corn starch) had significantly ($p<0.05$) higher protein than T2 (mackerel), suggesting that not all fish species have higher protein than livestock meats; thus quality of protein depends on the meat type. Although high protein contents were shown in catfish (T3), the protein contents for the two fishes were within the normal range of values of protein in fish (1525%) (Huss, 1995). The differences in protein contents of the fish sausages could be due to differences in species, feed availability, sexual maturity, spawning and season of catching (Oduor-Odote and Kazungu, 2008). The presence of high level of ash in T3 and T2 indicates that the total inorganic mineral contents in fish frankfurter are high due to the ability of fish to absorb certain trace elements from surrounding water (Tacon, 1992).

The results obtained for ash contents are within the ranges recorded by Burgaard and Jorgensen (2011).

Other studies ((Tozer, 2001) have reported ash values in the range of 1.05 - 1.29 %, which are lower than ranges found in this study (3.7- 5.92 %). The proximate composition of fish is species specific (Shearer, 1994), so it is logic to see differences between the examined fish sausages. However all values were within the normal ranges of proximate composition (water 66-84%, protein 15-25 % and fat 0.1 -24 %) (Huss, 1995).

The amount of carbohydrate in fish muscle is generally small to be of any significance in any balanced diet (FAO, 1985). Fish is usually less than 1% but 2-5% can be obtained in fatty species and striated muscle where carbohydrate occurs as glycogen and as part of the chemical constituents of nucleotides (FAO, 2013b). Carbohydrate was added to T2, T3 and T4 in the form of corn starch. T3 recorded significantly higher ($p<0.05$) carbohydrate

(3.51 %) followed by T4 (2.51%), T2 (2.24%) and T1 (1.89%). Meat muscle normally contains only traces of carbohydrates, in the form of sugars, sugar phosphates and glycogen (Wilson, 2002).

The chemical constituent can be as a source of free ribose after post mortem autolytic changes (FAO, 2013b). Therefore the high carbohydrate reported in this experiment could be due partially to the 1.2% corn starch added to treatments T2, T3 and T4. However T1 which had no corn starch added during product formulation recorded 1.18% carbohydrate.

4.5 Sensory Parameters

Table 4.3 shows the sensory parameters determined during the study. T1 and T4 recorded (higher $p<0.05$) values in all the sensory parameters tested, and T3 was also higher $p<0.05$) than T2 for all sensory attributes. It could be deduced that most of the panelists generally preferred pork sausages to fish sausages. According to (FAO, 2007), every meat product has its typical smell and taste and also different animal species have different tastes. (FAO, 2007) further stated that pH is important for the taste and flavour of meats. Lawrie and Ledward (2006) also indicated that high pH improves juiciness of products. Relating the pH (Table 4.0) to the juiciness, flavour and taste of sausages

(Table 4.3) in this research, it was observed that the result supports Lawrie and Ledward (2006). T1 and T4 recorded higher pH values indicating juicier products, which reflected in the sensory scores for juiciness of these sausages, compared to T2 and T3 which had lower pH values. Moreover, appearance, juiciness, texture, taste and overall acceptability of T4 (negative control = pork with corn starch) was not significantly different ($p<0.05$) from T1 (positive control = pork sausages without corn starch). Brown and Zayas (1990) have observed similar findings with the use of meat extenders in general.

Table 4.3 Sensory attributes of pork and fish frankfurters

							Overall
Week 1	6.93[a]	6.84[a]	6.67[a]	6.77[a]	6.60[a]	6.61[a]	6.89[a]
Week 6	6.93[a]	6.43[b]	6.39[b]	6.29[b]	6.19[b]	6.33[a]	6.4[b]
SEM	0.11	0.12	0.12	0.13	0.17	0.12	0.12
F.pr.	0.9919	0.0124	0.0833	0.0079	0.0180	0.1138	0.0064
Interaction							
Source	**Appearance**	**Flavour**	**Juiciness**	**Taste**	**Mouthfeel**	**Texture**	**acceptability**
Frankfurter- type							
T1	7.71[a]	7.45[a]	7.30[a]	7.58[a]	7.37[a]	7.28[a]	7.59a
T2	5.26[c]	5.42[c]	4.99[c]	4.89[c]	4.86[c]	5.22[c]	4.93c
T3	7.02[b]	6.36[b]	6.52[b]	6.34[b]	6.25[b]	6.43[b]	6.58b
T4	7.74[a]	7.31[a]	7.29[a]	7.30[a]	7.09[a]	6.94[a]	7.54a
SEM	0.15	0.16	0.16	0.18	0.17	0.17	0.17
F.pr.	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Storage							
T1 x Week 1	7.76	7.60	7.38	7.69	7.38	7.40	7.71
T1 x Week 6	7.66	7.30	7.23	7.48	7.36	7.16	7.48
T2 x Week 1	5.07	5.73	5.24	5.31	5.24	5.33	5.33
T2 x Week 6	5.45	5.11	4.73	4.48	4.49	5.12	4.53
T3 x Week 1	7.18	6.67	6.62	6.73	6.67	6.60	6.91
T3 x Week 6	6.86	6.05	6.41	5.95	5.84	6.25	6.25
T4 x Week 1	7.73	7.36	7.42	7.36	7.11	7.09	7.60
T4 x Week 6	7.75	7.27	7.16	7.25	7.07	6.80	7.48
SEM	0.21	0.23	0.23	0.26	0.24	0.24	0.23
F.pr.	0.4046	0.5791	0.8694	0.3534	0.1801	0.9934	0.4059

Superscript [abc] within columns are significantly different (<0.05)

Scale 1= dislike extremely, 2= dislike very much, 3= dislike moderately, 4= like slightly, 5= neither like nor dislike, 6= like slightly, 7 = like moderately, 8= like very much and 9= like extremely.

TxN= interaction between treatment and storage duration

T1= pork frankfurter without corn starch, T2= mackerel frankfurter, T3 = catfish frankfurter and T4 = pork frankfurter with corn starch

Sausages frozen for six weeks had lesser values in all attributes compared to week one of storage. This results support a report by Barroso *et al*., (1998), that during frozen storage meat muscle can denature and aggregate especially the myofibrillar proteins. These changes result in altered functional properties, changed textural attributes and reduced water holding capacity and juiciness. The result is a hard, dry and fibrous product with a reduced eating quality. Even though there were lesser values in week six than week one, no significant difference ($p>0.05$) were observed in the various treatments. Tacon (1992) reported that, quality of many foods depends on the concentration and type of minerals they contain including their taste, appearance, texture and stability. Thus all the sausage samples can be stored frozen for six weeks without any effects on sensory characteristics.

4. 6 Essential Amino Acid Contents of Fish (Mackerel and Catfish) and Pork Table 4.4 shows the levels of essential amino acids recorded in raw fish and pork used in this study. Aspartic acid, glutamic acid, arginine, methionine and threonine levels in catfish were significantly higher ($p<0.05$) than those of raw pork and mackerel. Catfish and mackerel recorded higher values in most of the amino acids in both raw product and their frankfurters (T2 and T3) than pork (Table 4.4.1). This supports report on essential amino acids in pork, fish and beef by FAO (2013b). The low levels of Aspartic acid, glutamic acid, methionine and leucine in pork corresponded with T4 (pork sausage). However, T2 recorded the highest levels in most of the amino acids analyzed. This indicates that fish is an excellent source of protein (FAO, 2012). The high levels of the various amino acids in the fishes and their frankfurters show that the protein in both raw fish and its frankfurter sausage are of higher quality than in raw pork and its frankfurtertype sausage. According to Keith (2002), high-quality proteins contain a lot of the essential amino acids.

Table 4.4.1 Essential Amino Acids Content of Fish (Mackerel and Catfish) and Pork

Amino acid (ppm)	Pork	Mackerel	Catfish	SEM
Aspartic Acid	2.02c	2.71b	3.73a	0.0037
Glutamic Acid	0.15c	0.33b	0.40a	0.0019
Histidine	0.65b	1.94a	0.17c	0.0009
Arginine	13.21b	12.91c	13.29a	0.0011
Valine	0.04a	0.04a	0.04a	0.0012
Methionine	0.11c	0.24b	0.43a	0.0011
Isoleucine	NT	0.04a	0.03b	0.0002
Phenylalanine	0.22a	0.02c	0.05b	0.0020
Threonine	0.21c	0.34b	0.42a	0.0006
Leucine	NT	0.08a	0.03b	0.0009

Superscript [abc]across rows are significantly different ($p<0.05$); NT= Not detected

The FAO (2013b) reported of the levels of essential amino acids in some animal protein sources, including fish. Lauritzen (1992) also reported that efficiency or degree to which dietary proteins can be used for building parts of the human body is determined principally by the type and relative amounts of amino acids present in the particular protein molecule. From this it could be deduced that the proteins in fish especially raw catfish and mackerel (and their frankfurters) could be more efficient in body building. FAO (2013b) reported similar results, high Methionine and isoleucine prevents excess fat buildup. Methionine helps relieve or prevent fatigue because it reduces histamine release, and may be useful in some allergy cases Keith (2002).

Table 4.4.2 Essential Amino acid contents of pork and fish sausages

Amino acid (ppm)	T1	T2	T3	T4	SEM
Aspartic Acid	4.08^{a}	3.05^{b}	2.37^{c}	2.03^{d}	0.00128
Glutamic Acid	0.52^{c}	1.05^{a}	0.78^{b}	0.41^{d}	0.40667
Histidine	1.67^{a}	0.00^{c}	NT	1.55^{b}	0.00193
Arginine	12.76^{a}	12.67^{c}	12.67^{c}	12.71^{b}	0.00157
Valine	0.07^{c}	0.69^{a}	0.52^{b}	0.07^{c}	0.00239
Methionine	0.27^{b}	0.70^{a}	0.70^{a}	0.08^{c}	0.00176
Isoleucine	0.03^{d}	0.87^{a}	0.59^{b}	0.26^{c}	0.00008
Phenylalanine	0.11^{a}	0.02^{c}	0.09^{b}	0.01^{d}	0.00075
Threonine	0.76^{d}	1.45^{a}	1.21^{b}	1.06^{c}	0.00341
Leucine	0.19^{c}	0.59^{a}	0.26^{b}	0.18^{d}	0.00305

Superscript [abc]across rows are significantly different ($p<0.05$); NT= Not detected

T1= pork frankfurter without corn starch, T2= mackerel frankfurter, T3 = catfish frankfurter and T4 = pork frankfurter with corn starch

4.7. Cations and Anions in Meats and Sausages

Figure 4.0 shows the results for various cations and anions determined in raw fish (catfish and mackerel) and pork. In all, a total of nine ions comprising of five cations (Magnesium, sodium, potassium, calcium, and ammonium) and four anions (Sulphate, flouride, chloride, and nitrate) were recovered in the raw meats. Mackerel and catfish recorded higher values in most of the ions determined (except ammonium, potassium and magnesium). However, raw pork which was low in most of the ions recorded higher mineral deposits than mackerel when they were processed (Fig 4.1 and Fig. 4.2).

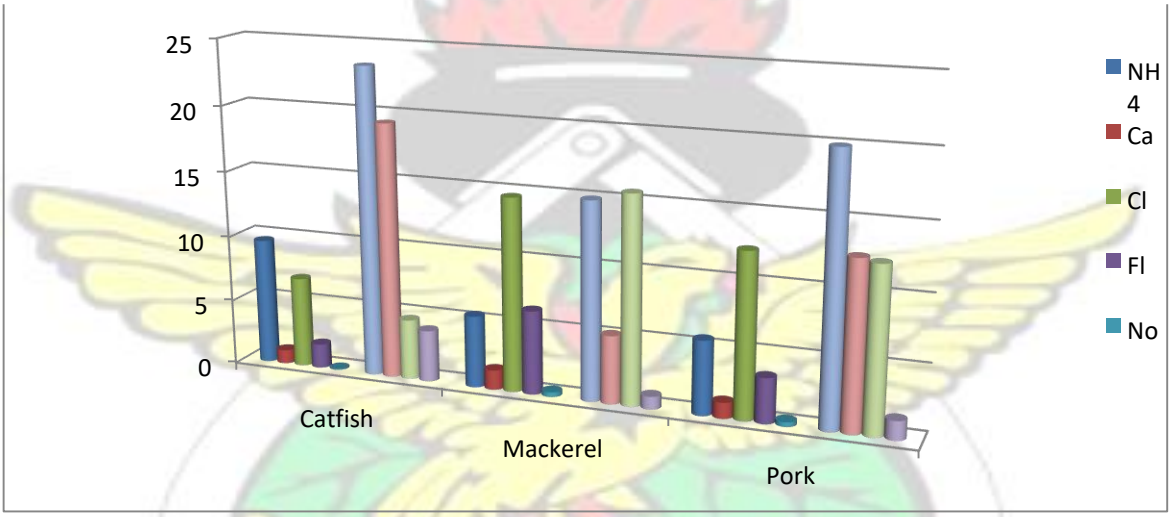

Figure 4.1 Ions (ppm) in raw meat

Serenius *et al.* (2006) reported that fish is a good source of vitamins B (thiamin, riboflavin and vitamin B_{12}), vitamins A and D as well as minerals such as calcium, phosphorous and iron. This indicates that the presence of other ingredients and or the processing method used could affect ion composition in a product. It is also important to know that mineral content of meat during processing can affect the physiochemical properties (Tacon, 1992). Low levels of calcium were observed in the frankfurters than their raw meats, in all treatments. Also, higher levels of NH_4^+ were observed for all the raw meats (pork, catfish and mackerel) but lesser values were recorded when processed into (frankfurters).

However, high values were reported for chloride in both raw meat and frankfurters in all the treatments.

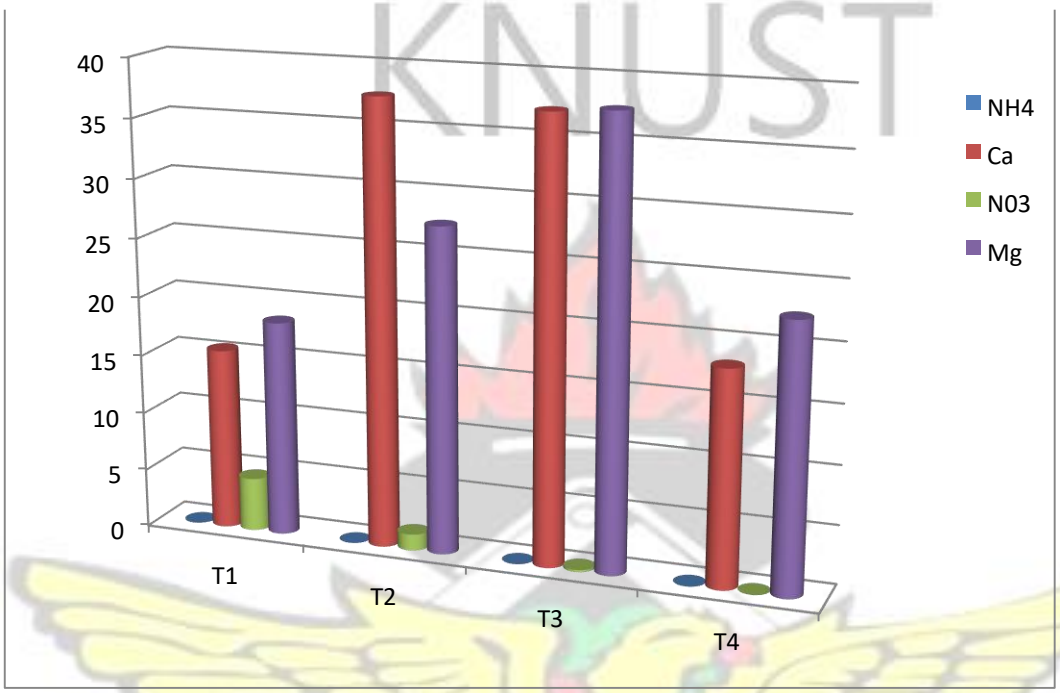

Figure 4.2 Levels of Mg^{2+}, $N0_3^-$, Ca^{2+} and NH_4^+ determined in Sausages

Sulphate values were also higher in raw meat than their frankfurters. Tacon (1992) reported that, the eating quality of food/meats including taste, texture, appearance and stability depends on the concentration of mineral. The results obtained in this study contradict (Tacon, 1992) because though the fish frankfurters were richer in most minerals (Ca^{2+}, Cl^-, K^+, and Na^+) their sensory acceptability was lower compared to pork sausages (Table 4.3). It is important to note that phosphate was not detected in all the raw meats used in the study but was recovered in the frankfurters because it was added in the form of polyphosphate during product formulation (Table 3.0).

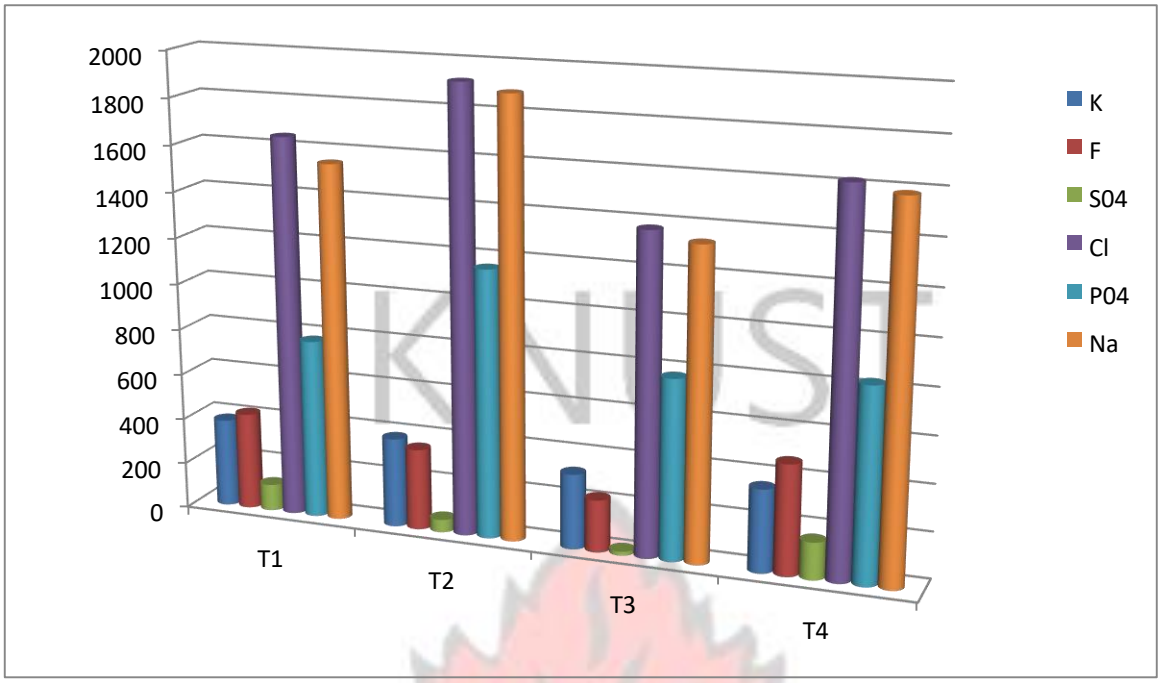

Figure 4.2 Levels of Na^{+}, $P0_4^{3-}$, Cl^{-}, $S0_4^{2-}$, F^{-} and K^{+} determined in Sausages

4.8 Microbial Counts and Isolation of *E. coli*

Tables 4.5, 4.5.1 and 4.5.2, show Total Viable Count (TVC) in minced meats, raw batters and frankfurters, respectively. The TVCs in all the analysis were within accepted limits (pork= 10^7 and fish= 10^6 cfu/g) as recommended by ICMSF (2002). Though the TVCs observed were within accepted limits, minced pork recorded higher counts numerically (2.5×10^5 cfu/g) than mackerel (1.9×10^5 cfu/g) and catfish (1.8×10^5 cfu/g). The low TVC in minced catfish and mackerel could be as a result of high ash contents (Table 4.2). Tacon (1992) reported that high mineral contents can sometimes retard the growth of certain microorganisms.

Table 4.5. Total Viable Count (TVC) for minced meat

Type of minced meat	TVC cfu/g	Recommended TVC (cfu/g) (ICMSF, 2002)
Pork	2.5×10^5	10^7
Mackerel	1.9×10^5	10^6
Catfish	1.8×10^5	10^6

TVC obtained after chopping minced meats into batter were also within accepted limits by ICMSF (2002). The observed TVC values ranged from 4.4×10^4 cfu/g (T3) to 2.5×10^5 cfu/g (T1). There was a 10^- fold reduction (10^5 to 10^4) in TVCs of minced mackerel and catfish after batter production.

Table 4.5.1 Total Viable Count (TVC) for Raw Meat batter

Type of frankfurter	TVC cfu/g	Recommended TVC (cfu/g) (2002)
T1	2.5×10^5	10^7
T2	9.9×10^4	10^6
T3	4.4×10^4	10^6
T4	1.1×10^5	10^7

T1= pork frankfurter without corn starch, T2= mackerel frankfurter, T3 = catfish frankfurter and T4 = pork frankfurter with corn starch

Also the addition of non meat ingredients (spice mix, water, phosphate, corn starch) may have reduced microbial population in raw fish batter. Sachindra *et al.* (2005) reported that micro-could organisms could gain access into sausage from meat, spices, and other ingredients, from environment, equipment, and handlers during processing. Thus the observed 10^- fold reduction in TVCs of the fish based batter is not in conformity to the assertion by Sachindra *et al.* (2005). Processing minced pork to obtain raw pork batter (T1 and T4) (Table 4.5.1) did not change the microbial counts (Table 4.5).

Table 4.5.2 Total Viable Count (TVC) of sausages during frozen storage

Storage (week)	Type of frankfurter TVC (cfu/g)				Recommended TVC (cfu/g) (ICMSF 2002)			
	T1	**T2**	**T3**	**T4**	**T1**	**T2**	**T3**	**T4**
1	1.7×10^5	1.9×10^5	7.1×10^4	1.2×10^5	10^6	10_5	10_5	10^6
2	7.6×10^4	2.3×10^4	9.0×10^4	6.0×10^3	10^6	10_5	10_5	10^6
3	5.0 $\times 10^3$	2.0×10^3	2.2×10^4	7.3×10^3	10^6	10_5	10_5	10^6
4	2.7 $\times 10^4$	3.7×10^4	8.0×10^3	7.0×10^2	10^6	10_5	10_5	10^6
5	8.4 $\times 10^4$	1.3×10^5	4.4×10^4	9.3×10^4	10^6	10_5	10_5	10^6
6	9.0 $\times 10^4$	1.3×10^5	3.0×10^5	2.6×10^4	10^6	10_5	10_5	10^6

Table 4.5.2 is the TVC for frankfurters during frozen storage. The observed counts were all within acceptable limits; since 10^6cfu/g is the maximum permissible level for aerobic plate counts in meat products ICMFS (2002). Thus, all the frankfurters analysed over the six weeks of frozen storage can be classified as acceptable for human consumption. Pierard *et al*. (1999) reported that most pathogenic bacteria grow better in meats when the pH ranged from 6 to 8. This study supports Pierard *et al*. (1999) because the pH values recorded in all the frankfurters ranged from 5.41 to 5.78 (Table 4.0).

E. coli were not detected in this study at all stages of sampling (Table 4.6. to Table 4.6.3). In fact, no growth was observed during week five and six of frozen storage. **Table 4.6.0 Isolation of organism (*E. coli)* in minced meat**

Minced meat	***E. coli***
Pork	Not detected
Mackerel	Not detected
Catfish	Not detected

De Roever (1998) indicated that, the presence of *E. coli* in food is an indicator of crosscontamination. Minced meat, raw batter and frankfurters made from pork, mackerel and catfish from this study reported no *E.coli* prevalence.

Table 4.6.1 Isolation of organism *(E. coli)* in raw batter

Minced meat	***E. coli***
T1	Not detected
T2	Not detected
T3	Not detected
T4	Not detected

This could be attributed to the fact that, the production process of cooked meats which included a heating/smoking step was sufficient to eliminate any *E. coli* and other pathogens present. Tacon, (1992) reported of presence of *E. coli* in pork sausages. Several authors found that *E. coli* O157:H7 was associated with derived retail meat products (Chinen *et al*., 2001; Chapman *et al.,* 2001). Of the 1750 ground pork and fish samples analyzed, 20 (1.1%) were positive for *E. coli* O157 (Samadpour *et al.,* 2006).

Table 4.6.2 Isolation of organism (*E. coli*) in frankfurter during frozen storage (week 1-week 3)

Sausage week one	***E. coli***
T1	Not detected
T2	Not detected
T3	Not detected
T4	Not detected
Sausage week two	***E. coli***
T1	Not detected
T2	Not detected
T3	Not detected
T4	Not detected
Sausage week three	***E. coli***
T1	Not detected
T2	Not detected
T3	Not detected
T4	Not detected

The use of curing salt (containing nitrite) in the formulation of the frankfurter could also have contributed to eliminating pathogenic organisms in the products during storage because nitrite is effective as antimicrobial addictive in meat systems.

Table 4.6.3 Isolation of organism *(E. coli*) in frankfurter during frozen storage (week 4-week 6)

Sausage week four	***E .coli***
T1	Not detected
T2	Not detected
T3	Not detected
T4	Not detected
Sausage week five	***E. coli***
T1	Not detected
T2	Not detected
T3	No growth
T4	No growth
Sausage week six	***E. coli***
T1	No growth
T2	No growth
T3	No growth
T4	No growth

**E. coli* was absent in spice mix

The spices used in this experiment had no *E. coli* presence. It has been suggested that several factors (environmental factors) may contribute to the presence of pathogens, including poor handling, poor hygiene practices, cross-contamination from food handlers and storage conditions (Chinen *et al*., 2001). This indicates that sufficient hygienic measures for microbial control were put in place during the manufacturing process of the study.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Catfish frankfurter was higher in protein compared to mackerel and pork sausages. Low fat contents were also recorded in catfish sausage. Most of the essential amino acids and minerals were higher in fish (mackerel and catfish) and their sausages than pork.

Raw mackerel recorded the highest values in most of the ions determined (except ammonium, potassium and magnesium). However mackerel frankfurter and pork frankfurter (without corn starch) recorded (numerically) higher ion deposits when the raw products were processed.

Pork frankfurter (with corn starch) was able to hold much water than pork frankfurter (without corn starch), mackerel frankfurter and catfish frankfurter. There was no difference in cooking yield between catfish frankfurter and pork frankfurter (with corn starch).

From the sensory test, most of the panelists preferred pork frankfurters to fish frankfurters.

Microbes were present in both fish and pork sausages but lesser Total Viable Counts (TVC) were recorded as frozen storage weeks increased. In fact TVCs recorded were all within the accepted limits as recommended by (International Commission on Microbiological Specification on Food). *E. coli* was not detected in all raw ingredients as well as their respective frankfurter samples. This indicates that sausages were produced under good hygienic conditions and proper handling practices were adhered to at all stages of processing.

It can therefore be concluded that catfish frankfurter has high crude protein, less fat, appreciable amount of ions and has high cooking yield.

5.2 Recommendation

It is recommended that, further work could be done to improve on flavour, taste, mouthfeel, appearance, juiciness and texture of fish frankfurters. Pork could be partially replaced with fish in frankfurters in order to improve functional and binding properties.

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7.0 APPENDICES

7.1 Chemical Analysis

7.1.1 Moisture content (Microwave Oven Drying)

The determination of the moisture content (or water content) was done by drying an appropriate amount of the sample. The difference in weight between the fresh and dried samples represents the water content.

Procedure:

- ✓ Prepared sausage sample was defrosted.
- ✓ The oven preheated
- ✓ The beakers were dried by heating them in a microwave oven for one minute.
- ✓ The heating time necessary to completely dry the samples in the microwave oven was determined.
- ✓ About 10 grams of sample was weighed into beaker. The samples were then placed in the preheated oven and spaced at equal distances around the turntable at 135^0C for 2 hours.
- ✓ The samples were cooled in a desiccator and accurately weighed.
- ✓ Drying was repeated until constant weight was obtained.

Expression of results:

$$\%\ Moisture\ = \frac{(Fresh\ weight\ of\ sample - Oven\ dried\ weight\ of\ sample)}{Fresh\ weight\ of\ sample} \times 100$$

7.1.2 Protein content

Nitrogen content is estimated by the Kjeldahl method which is based on the determination of the amount of reduced nitrogen present in a sample (Slack 1987), and protein calculated as total Nitrogen × 6.25 (%). The various nitrogenous compounds were converted into ammonium sulphate by boiling with concentrated H_2SO_4. The ammonium sulphate formed was decomposed with an alkali (NaOH) and the ammonia liberated was absorbed in excess of neutral boric acid solution and titrated with a standard acid (Islam et al., 2010).

7.1.2.1 Digestion stage

Each sample (1.25g) was weighed and put in a Kjeldahl flask. Three spatulas full of a mixture of copper sulfate and sodium sulfate was added to each sample and mixed. 25 ml of concentrated H_2SO_4 was added to each sample and the flask was placed in an inclined

position on a Kjeldahl digestion system and heated slowly until bubbling ceased and the resulting solutions is clear. The digested samples were diluted with 80 ml of distilled water after cooling. Samples will then be transferred in to a distillation apparatus.

7.1.2.2 Distillation stage

Twenty five (25) ml of 2% boric acid solution was pipette into a 250 ml conical flask and 2 drops of mixed indicator was added. The conical flask with its content was placed under a condenser such that the tip of the condenser is completely immersed in the solution. About 50ml of 40% NaOH was added to the test solution in the decomposition flask. The stop cork on the steam trap outlet was closed, which forced the steam through the decomposition chamber and in the process drove the liberated ammonia into the collection flask. Distillation was continued until 150ml of distillate is collected.

7.1.2.3 Titration

The distillate was titrated with 0.1 M HCL in the micro burette on the zero mark. The same procedure was followed for the blank titration. The titre values of the duplicated samples was recorded and the percentage protein calculated as follows:

$$\%\ Nitrogen = \frac{(Sample\ titre - blank\ titre \times\ Normality\ of\ HCL\ X\ 14 \times Volume\ made\ up\ of\ Digestion\ \times 100)}{Aliquot\ of\ Digestion\ taken\ \times Weight\ of\ Sample\ taken\ \times\ 1000}$$

% protein = % Nitrogen x 6.25

7.1.3 Fat determination

The Soxhlet apparatus was used for the determination of fat content. Two grams of each sample was weighed and put into a 22 x 80mm folded filter paper. A 250ml round bottom flask was weighed. 150ml of petroleum spirit were added to the flask. A quick fit condenser was connected to the Soxhlet extractor and refluxed for 2hours on low heat. The flask was removed and evaporated on steam bath. The flask and its contents were then cooled to

room temperature in a desiccator after which it was weighed and percentage fat calculated as:

$$\%\,Fat = (Weight\ of\ Sample - Weight\ of\ defatted\ /(Original\ weight\ of\ Sample) \times 100$$

7.1.4 Ash determination

- ✓ Two grams of the defatted sample is placed in a constant weight porcelain crucible with cover.
- ✓ The crucible is then placed in a muffle furnace, and at a temperature of 600°C the sample is ignited for two hours.
- ✓ After ignition the crucible was placed in the oven to bring down the temperature for about 30 minutes, then cool in a dessicator for another 30 minutes and re-weighed.
- ✓ The sample will then be weighed to constant weight.

Computation:

$$\%Ash = \frac{\text{weight of crucible with cover+ash - weight of crucible with cover}}{\text{Originalweightof sample}} \times 100$$

7.2 Acidity (pH) measurement

The procedure according to Hack-Young *et al*., (2010) was used to determine the pH. The pH values were obtained by directly inserting the pH meter into different portions of sausage sample. Five grams of fresh sausage paste samples from each treatment was mixed thoroughly with 50ml distilled water in centrifuge tube. The solution obtained was allowed to settle for 15minutes, after, the pH meter was lowered into the sample and pH recorded. Each sample was replicated thrice to ensure accuracy and also minimize errors.

7.3 Water Holding Capacity (WHC)

A 10g sample of sausage raw batter and finish product were placed in a glass jar and heated at 90ºC for 10 minutes in a water bath. It was then removed, cooled to room temperature and then centrifuged for 10 minutes at 10,000 rpm at 4ºC. The sample was reweighed. The WHC was then calculated as follows:

$$\%\,WHC = \frac{B-A}{B} \times 100\%$$

Where B = Initial weight of sample

A = weight of sample after centrifuging

7.4 Amino Acid Determination

Amino acids were determined in samples by ion-exchange chromatography of the acid hydrolyzed protein. Both minced and sausage samples of pork, mackerel, catfish and their sausages were analyzed.

Amino acid standards (2.5 moles/L) were purchased from Sigma-Aldrich, 2,4-dinitrofluorobenzene (DNFB), HPLC grade methanol and acetonitrile were purchased from MES Chemicals(Ghana). Other chemicals used were of analytical reagent grade.

Water used for preparation of reagents was double distilled water.

Apparatus

HPLC system (Varian) consisting of a Varian ProStar 210/215/218/SD-1 Pumps,Varian ProStar 325LC Detector. Galaxie software for data processing and Genini µL C18 110A 150 * 4.60 mm 5 micron 257052-7 analytical column was used for separation.

The mobile phase consisted of Mobile Phase A (0.02 mol/L Na2HPO4 + 0.02 mol/L NaH2PO4) and Mobile Phase B (Methanol: Acetonitrile=10: 90(v/v) mixed in a 70:30 ratio with a flow rate of 1.3 mL/min. The analysis was carried out at room temperature.

7.4.1 Standard Solution/Sample Preparation

Dilute solutions of the standard Amino Acids were then prepared by pipetting 20, 40, 60, 80 and

100 ㄐ L of the stock solution into a 15 ml centrifuge tube and diluted with 20 ml 0.2 M NaHCO3.

1 mL of 1% DNFB (a 1 mL portion of DNFB was dissolved in 100 mL of methanol). These testtubes were then placed on a water bath (60^{o}C) for 40 minutes. All derivatization reactions were stopped by addition of 0.5 mL of 1 mol / L HCL. The resulting derivative was then filtered.

All the samples were treated in the same manner as described above for standard. That is, 1 ml of each sample were placed in a 15 ml centrifuge tube, 2 ml of NaHCO3 added and the resulting mixture derivatized by adding the 1 % DNFB(1-fluoro-2,4-dinitrobenzene). 20 µL of each standard and sample were then injected into the HPLC system.

7.5 IONS DETERMINATION IN MEAT SAMPLES

7.5.1 Chemicals and Reagents

Double distilled water, sodium Chloride, ammonium chloride, sodium nitrate, calcium chloride, sodium phosphate, magnesium Sulphate, Potassium bromide and potassium fluoride.

7.5.2 Chromatographic conditions

861 Advanced Compact IC (Metrohm) consisting of a peristaltic pump and a conductivity detector. Metrohm software for data processing and Metrosep A Supp 5
100/4.0 mm analytical column was used for separation of anions whiles Metrosep C4 150 150/4.0 mm analytical column for cations separation. The Eluent for anion analysis consists of a mixture of 1. 0 mmol/L of sodium hydrogen carbonate and 3.2 mmol/L of sodium carbonate. The eluent for cation analysis consists of 2 mmol/L of nitric acid. The analysis was carried out at room temperature.

7.5.3 Anions

- 500 ppm of standard anion solution consisting of Fluoride, Chloride, Bromide, Nitrate, Phosphate and sulphate were prepared.
- Serial dilutions (10, 20, 30, 40 and 50 ppm) of the ions were then performed by diluting 2, 4, 6, 8 and 10 ml of the stock solution in 100 ml volumetric flask and made to the mark with the double distilled water.
- The homogenized samples were filtered and the filtrate analysed for the presence of the desired ions.

7.6 Cost of Production

Catfish	**Mackerel**
Live weight =31Kg	Frozen weight= 12.75Kg
Weight after bleeding = 30.25 Kg	Boneless weight=6.7Kg
Boneless= 14.90kg	Head = 2.1Kg
Head = 7.0kg	Bones/skin= 2.34Kg

Gut content= 1.1 Gut content = 1.05kg

Bones/fins/skin= 7.25Kg Entrails (head+bones+ skin+ gut content) = 5.45Kg

Entrails (head+bones+ skin+ gut content)= 16.15Kg

$$Dressing\ \% = \ deboned\frac{weight}{weight}afterbleeding * 100$$

$$thus\ Catfish\frac{14.9}{3025} * 100 = \ 49.26\% \qquad Mackerel\frac{6.7}{12.75} * 100 * = 52.55\%$$

Cost per Kg for samples

$$Cost/kg = total\ ingredient\ cost(GH¢)/$$

$$Total\ kg\ obtained\ after\ producing\ each\ treatment\ (excluding\ casing, spice, labor\ and\ overheads)$$

T1 = GH¢ 30.19/4.5kg= GH¢ 6.70, 6.7*100/68.04= GH¢ 9.85

T2 = GH¢ 25.52/4.2kg= GH¢ 6.10, 6.10*100/79.07= GH¢7.72

T3 = GH¢ 33.39/4.5kg= GH¢7.42, 7.42*100/83.03= GH¢8.94

T4= GH¢ 30.67/4.7kg = GH¢6.53, 6.53*100/91.55= GH¢ 7.12

7.7 MICROBIOLOGY

7.7.1 Sterilization of Materials and Media Preparation

The materials used for this study were sterilized by appropriate methods to free them from microbial contamination. All the media used were sterilized at 121ºC for 15minutes in an autoclave and were prepared according to the manufacturers' instruction or standard methods for examination of water (APHA,1992). Culture media

generally used for the study were plate count agar, nutrient agar and Macconkey broth. All glass wares were sterilized in the hot air oven at 160ºC for two hours. The inoculating needle and wire were sterilized by flaming in the Bunsen burner until red hot, working surface were sterilized by the application of disinfectants/antiseptic solutions (95% ethanol).

7.7.2 Plate count Agar

The powder consists of tryptone 5.0g/l, yeast extract 2.5g/l glucose 1.0g/l and agar 12.0g/l (park scientific limited, Northampton uk).

20.5g of the media powder was weighed into 1 litre of distilled water. The solution was sterilized by autoclaving at 121^0C for 15 minutes. It was then cooled to 47ºC before used.

7.7.3 Macconkay broth

The composition of the media powder is 20g of peptone, 10g of lactose,5g of sodium chloride,0.075g neutral red and 5g of bile salt (oxoid limited, England)

40g of the media powder was added to 1 litre of distilled water and allowed to stand for 10 minutes.5ml of the solution was dispensed into test tubes and corked with cotton. Media in test tubes were sterilized by autoclaving at 121ºC for 15 minutes. The final pH of 7.4 ± 0.2 at temperature of 25ºC was reached.

7.7.4 Tryptophan broth

The media powder contains 10g of meat peptone, 1.0g of L-tryptonphan, 5g of sodium chloride (Scharlan chemie S.A, Barcelona, Spain)

16g of the powder was added to 1 litre of deionised water.5ml of the solution was dispensed into test tubes and then sterilized by autoclaving at 121ºC for 15 minutes.

Reagent

Kovac's reagent was used for indole test to test for the presence of *Escherichia coli*

7.7.5 Microbiological analysis

Total coliforms were estimated using a three-tube Most Probable Number (MPN) method according to standard procedures (Anon, 1992). Ten grams of each sample (spice, minced meat, batter and sausage) was placed in a stomacher bag and pulsified in 90ml of 0.9% Nacl MQ water for 30s using stomacher (pul-100E; Stuart scientific co. ltd.uk). Serial dilutions of up to 10^{-4}were prepared from 1ml of the stomacher bag content.

One milliliter of each dilution was inoculated in triplicate into 5ml of maconkay broth medium. Tubes showing acid and gas production after incubation for 24hrs at 44^0C was confirmed by transferring a drop of liquid from positive tubes into 5ml test tubes of tryptophan broth and incubated at 44^0C for 24hrs. A drop of Kovac's reagent was then added to the tubes of tryptophan broth. All tubes showing a red ring colour development after gentle agitation was recorded as positive for thermo tolerant (fecal) coliform.

Estimated count was obtained from MPN tables (APHA-AWWA-WEF, 2001) and result was expressed in colony forming unit per gram for spice, minced meat, batter and sausages stored in the refrigerator for six weeks using colony counter. Total viable cell was enumerated by placing 1ml volume of the same serial dilutions prepared for the thermo tolerant coliforms directly onto set plates of plate count agar. These were allowed to dry and then incubated for 24 hrs at 44^0c and 24hrs at 37^0c respectively.

7.7.6 Isolation of specific pathogen

7.7.6.1 *E. coli*

The test tubes containing macconkay broth were incubated at 44^{o}C for 24hrs .They were observed for a colour change from reddish (original colour) to yellowish for the presence of faecal coliforms and also recorded as positive.

E. coli was then tested for by inoculating 1ml of the culture into sterile test tubes containing 5ml of tryptophan broth. Test tubes containing tryptophan broth were labeled according to the number on each test tubes containing macconkay broth. It was then incubated at 44^{o}C for 24hrs.Trytophan broth tubes became turbid after incubation period.

7.7.6.2 Indole Test

Confirmatory test for the presence of *E.coli* was done by performing indole test. This was done by adding a drop of kovac's reagent into the tubes. The presence of red colour ring at a meniscus of the tryptopan broth in the test tube indicated that there was presence of *E.coli*.

7.8 Anova of Amino Acids (Mackerel, Catfish and Pork)

Variate: Valine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	2.222E-07	1.111E-07	0.02	0.975
Residual	6	2.667E-05	4.444E-06		
Total	8	2.689E-05			

Variate: Serine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	2.344E-01	1.172E-01	1.055E+06	<.001
Residual	6	6.667E-07	1.111E-07		
Total	8	2.344E-01			

Variate: Proline

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	1.570E-01	7.849E-02	1.413E+05	<.001
Residual	6	3.333E-06	5.556E-07		
Total	8	1.570E-01			

Variate: Cystine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	0.64297867	0.32148933	9394.17	<.001
Residual	6	0.00020533	0.00003422		
Total	8	0.64318400			

Variate: Phenylalanine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	0.00134867	0.00067433	58.36	<.001
Residual	6	0.00006933	0.00001156		
Total	8	0.00141800			

Variate: Methionine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	1.607E-01	8.036E-02	20663.06	<.001
Residual	6	2.333E-05	3.889E-06		
Total	8	1.607E-01			

Variate: Leucine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	1.062E-02	5.308E-03	2252.20	<.001
Residual	6	1.414E-05	2.357E-06		
Total	8	1.063E-02			

Variate: Arginine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	2.429E-01	1.215E-01	34160.28	<.001
Residual	6	2.133E-05	3.556E-06		
Total	8	2.429E-01			

Variate: Alanine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	0.0216667	0.0108333	48.75	<.001
Residual	6	0.0013333	0.0002222		
Total	8	0.0230000			

Variate: Isoleucine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	2.401E-03	1.201E-03	11026.50	<.001
Residual	6	6.533E-07	1.089E-07		
Total	8	2.402E-03			

Variate: Histidine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	5.045E+00	2.522E+00	8.731E+05	<.001
Residual	6	1.733E-05	2.889E-06		
Total	8	5.045E+00			

Variate: Glutamic_Acid

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	0.09208289	0.04604144	4228.30	<.001
Residual	6	0.00006533	0.00001089		
Total	8	0.09214822			

Variate: Aspartic_Acid

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	4.42800822	2.21400411	51890.72	<.001
Residual	6	0.00025600	0.00004267		
Total	8	4.42826422			

7.9 Anova of Ions (Mackerel, Catfish and Pork)

Variate: Fluoride

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	31.566	15.783	10.00	0.012
Residual	6	9.467	1.578		
Total	8	41.033			

Variate: Chloride

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	94.979	47.489	23.55	0.001
Residual	6	12.097	2.016		
Total	8	107.076			

Variate: Calcium

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	2	0.31547	0.15773	7.60	0.030
Residual	5 (1)	0.10373	0.02075		
Total	7 (1)	0.41740			

Variate: Threonine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	6.314E-02	3.157E-02	25831.91	<.001
Residual	6	7.333E-06	1.222E-06		
Total	8	6.315E-02			

Variate: Sulphate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	193.4160	96.7080	166.05	<.001
Residual	6	3.4944	0.5824		
Total	8	196.9104			

Variate: Sodium

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	299.17247	149.58623	5183.97	<.001
Residual	6	0.17313	0.02886		
Total	8	299.34560			

Variate: Ammonium

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	32.5622	16.2811	147.68	<.001
Residual	6	0.6615	0.1102		
Total	8	33.2237			

Variate: Magnesium

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	15.30127	7.65063	160.65	<.001
Residual	6	0.285730.04762			
Total	8	15.58700			

Variate: Potassium

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	108.59509	54.29754	746.41	<.001
Residual	6	0.43647	0.07274		
Total	8	109.03156			

Variate: Phosphate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	0	0		
Residual	6	0	0		
Total	8	0			

Variate: Nitrate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	0.149422	0.074711	18.27	0.003
Residual	6	0.024533	0.004089		
Total	8	0.173956			

7.10 Anova of Ions (T1, T2, T3 and T4)

Variate: Magnesium

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	619.3206	206.4402	241.05	<.001
Residual	8	6.8513	0.8564		
Total	11	626.1719			

Variate: Calcium

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	1280.729	426.910	290.43	<.001
Residual	8	11.759	1.470		
Total	11	1292.489			

Variate: Chloride

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	446210.35	148736.78	2908.65	<.001
Residual	8	409.09	51.14		
Total	11	446619.44			

Variate: Fluoride

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	104040.58	34680.19	873.54	<.001
Residual	8	317.61	39.70		
Total	11	104358.19			

Variate: Ammonium

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.0049000	0.0016333	2.02	0.190
Residual	8	0.0064667	0.0008083		
Total	11	0.0113667			

Variate: Phosphate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	303022.72	101007.57	2142.56	<.001
Residual	8	377.15	47.14		
Total	11	303399.86			

Variate: Potassium

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	7542.66	2514.22	103.10	<.001
Residual	8	195.09	24.39		
Total	11	7737.75			

Variate: Nitrate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	40.359267	13.453089	1672.92	<.001
Residual	8	0.064333	0.008042		
Total	11	40.423600			

Variate: Sodium

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	4.613E+05	1.538E+05	6.129E+05	<.001
Residual	8	2.007E+00	2.509E-01		
Total	11	4.613E+05			

Variate: Sulphate

Source of variation	d.f.	s.s.	m.s.	v.r	F pr.
Treatment	3	36283.430	12094.477	11223.79	<.001
Residual	8	8.621	1.078		
Total	11	36292.051			

7.11 Anova of Amino Acids (T1, T2, T3 and T4)

Variate: Threonine,CT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.74879933	0.24959978	7148.44	<.001
Residual	8	0.00027933	0.00003492		
Total	11	0.74907867			

Variate: Valine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.90087425	0.30029142	17578.03	<.001
Residual	8	0.00013667	0.00001708		
Total	11	0.90101092			

Variate: Proline

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	5.698E+00	1.899E+00	5.698E+06	<.001
Residual	8	2.667E-06	3.333E-07		
Total	11	5.698E+00			

Variate: Serine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	1.072E+02	3.573E+01	3.085E+06	<.001
Residual	8	9.267E-05	1.158E-05		
Total	11	1.072E+02			

Variate: Methionine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	8.850E-01	2.950E-01	31606.79	<.001
Residual	8	7.467E-05	9.333E-06		
Total	11	8.851E-01			

Variate: Phenylalanine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	2.193E-02	7.309E-03	4385.52	<.001
Residual	8	1.333E-05	1.667E-06		
Total	11	2.194E-02			

Variate: Alanine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	3.838284	1.279428	210.71	<.001
Residual	8	0.048576	0.006072		
Total	11	3.886860			

Variate: Arginine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	1.599E-02	5.329E-03	718.45	<.001
Residual	8	5.933E-05	7.417E-06		
Total	11	1.604E-02			

Variate: Aspartic_Acid

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	7.372E+00	2.457E+00	4.998E+05	<.001
Residual	8	3.933E-05	4.917E-06		
Total	11	7.372E+00			

Variate: Cystine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	8.930E-03	2.977E-03	11907.00	<.001
Residual	8	2.000E-06	2.500E-07		
Total	11	8.932E-03			

Variate: Glutamic_Acid

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	7.315E-01	2.438E-01	1.330E+05	<.001
Residual	8	1.467E-05	1.833E-06		
Total	11	7.315E-01			

Variate: Histidine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	7.80350558	2.60116853	2.329E+05	<.001
Residual	8	0.00008933	0.00001117		
Total	11	7.80359492			

Variate: Isoleucine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	1.215E+00	4.049E-01	2.314E+07	<.001
Residual	8	1.400E-07	1.750E-08		
Total	11	1.215E+00			

Variate: Leucine

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.33489700	0.11163233	4010.74	<.001
Residual	8	0.00022267	0.00002783		
Total	11	0.33511967			

7.12 Ions in meat samples

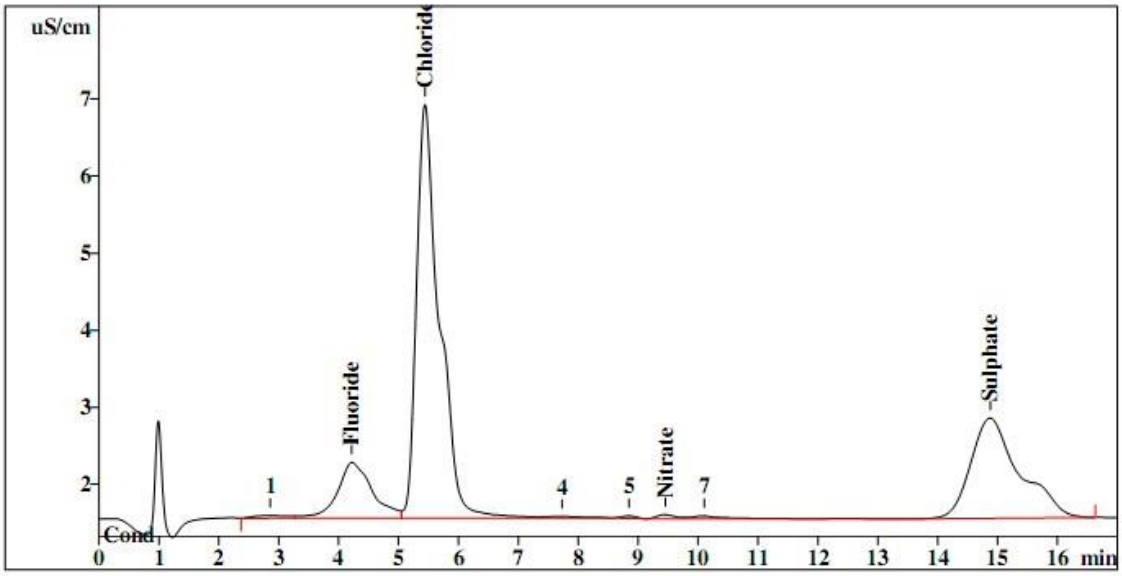

Figure 1 Anions in catfish

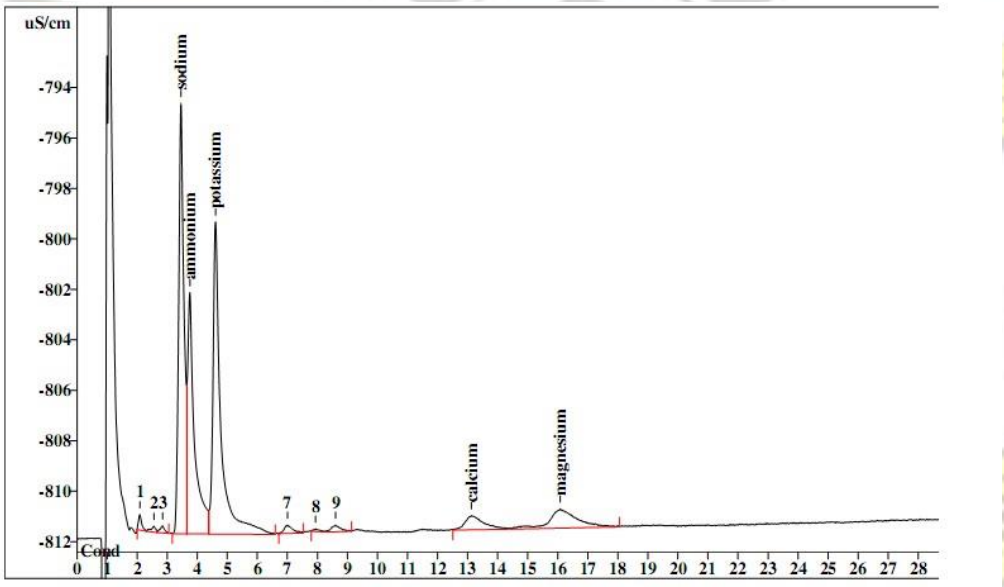

Figure 2 Cation in catfish

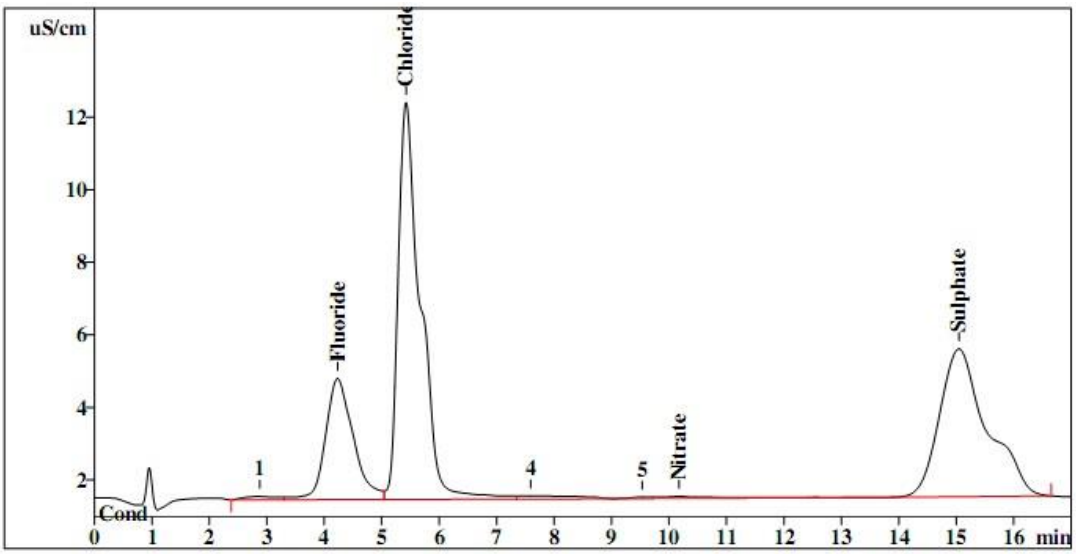

Figure 3 Anion in Mackerel

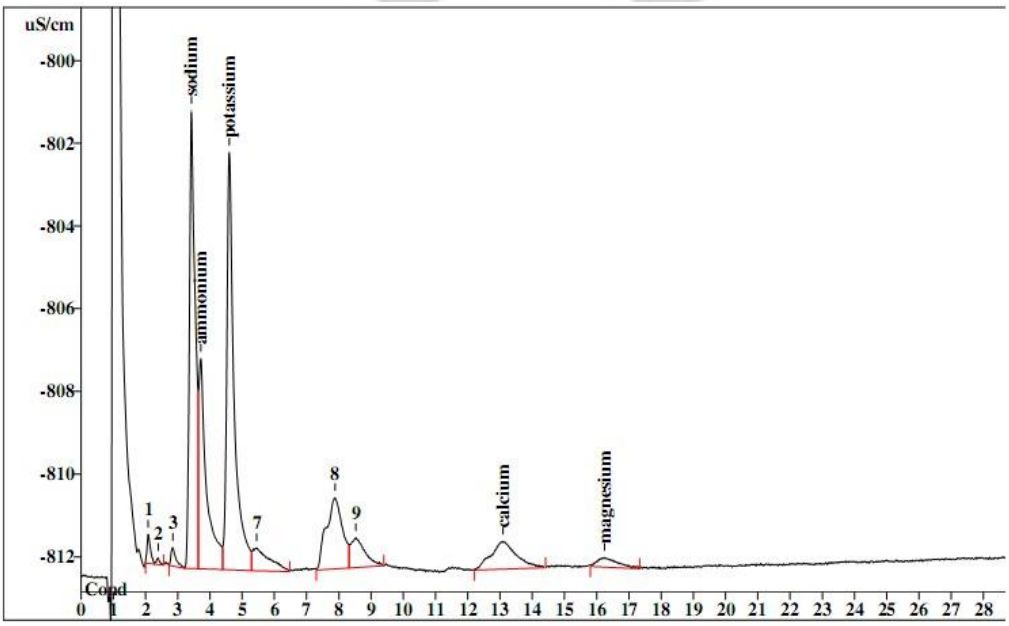

Figure 4 Cation in Mackerel

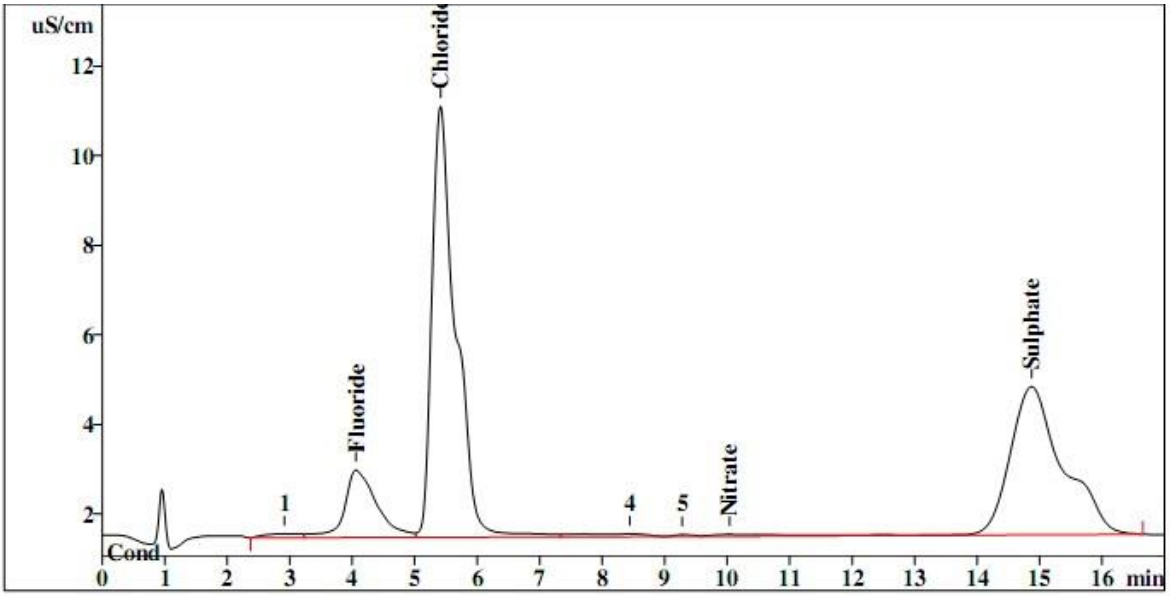

Figure 5 Anion in Pork

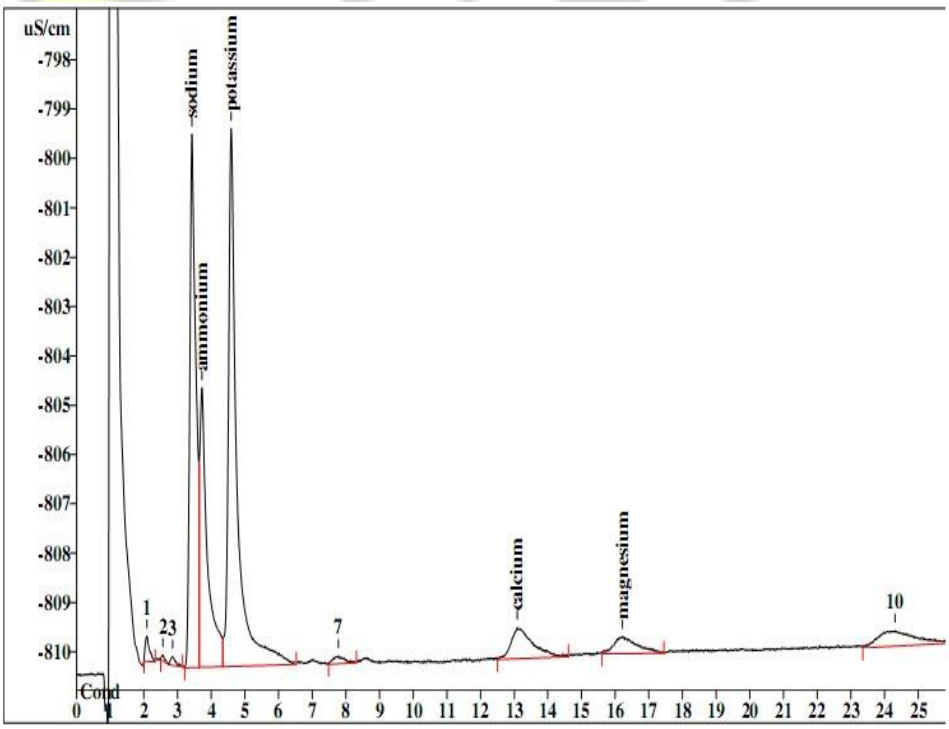

Figure 6 Cation in pork

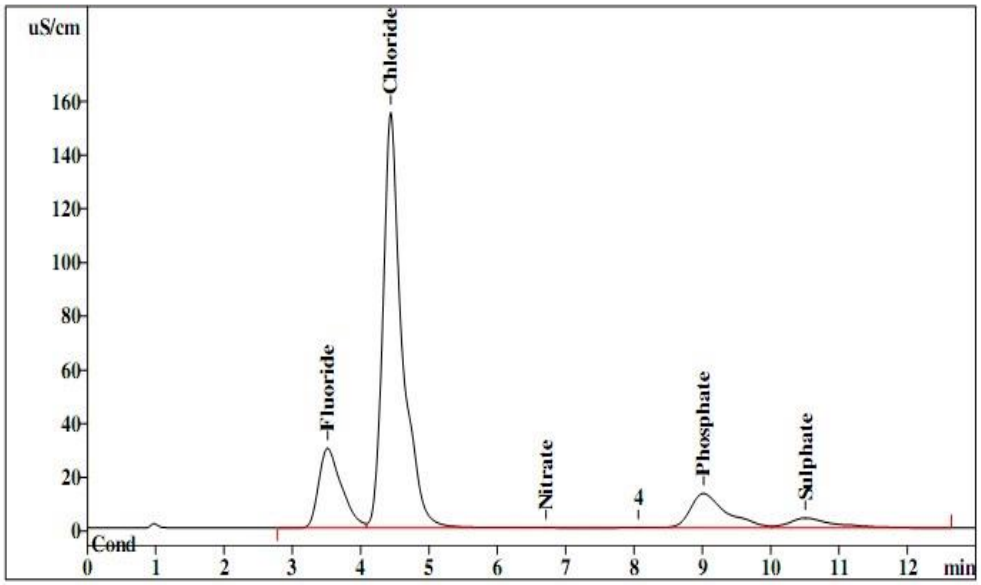

Figure 7 Anion in T1

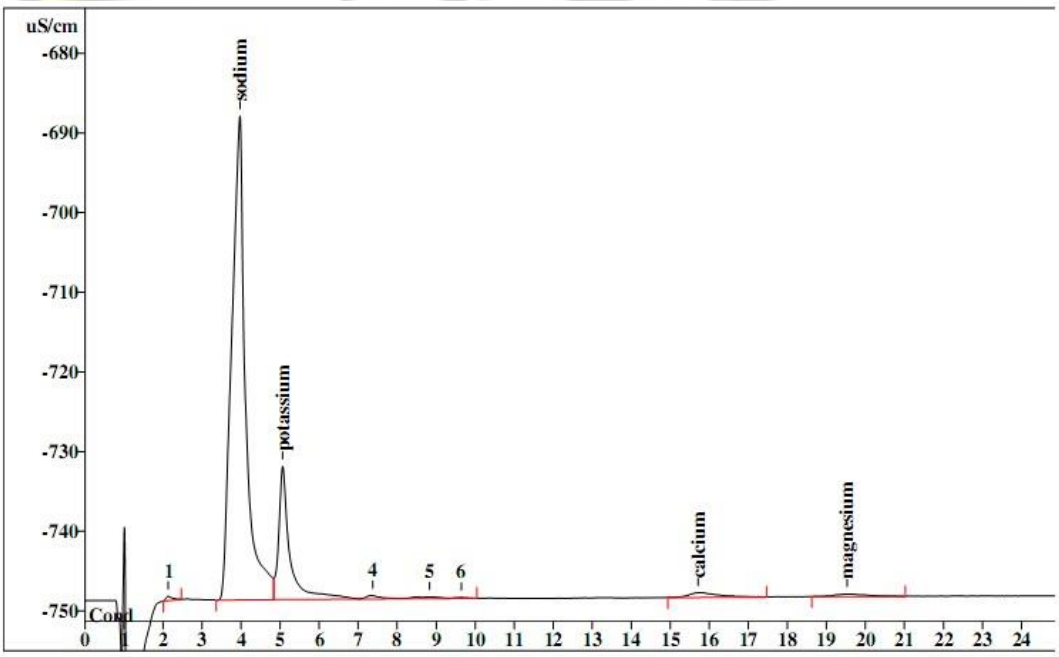

Figure 8 Cation in T1

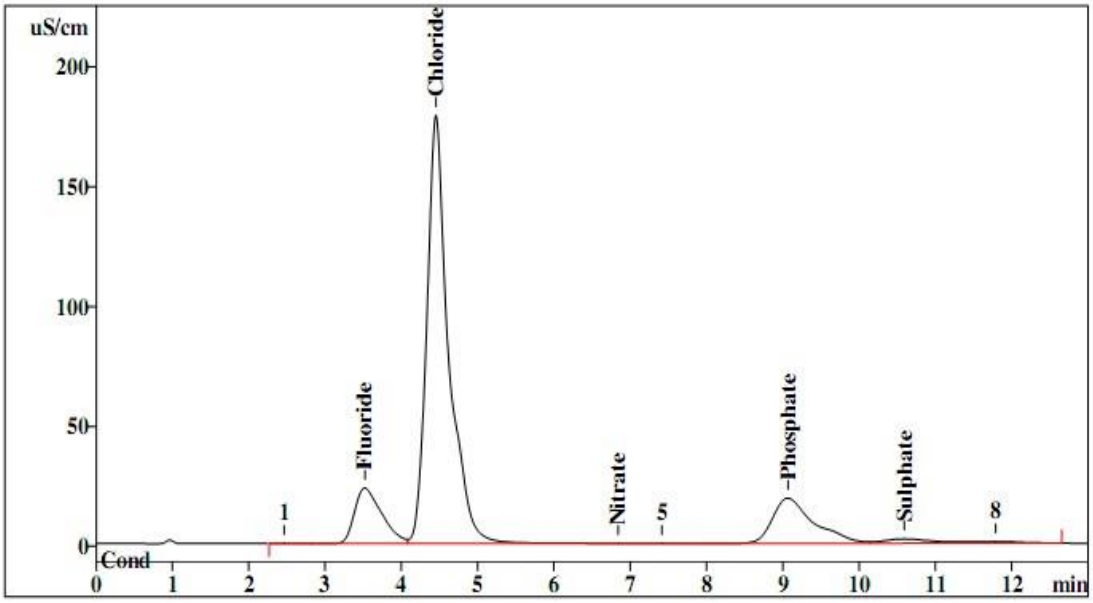

Figure 9 Anion in T2

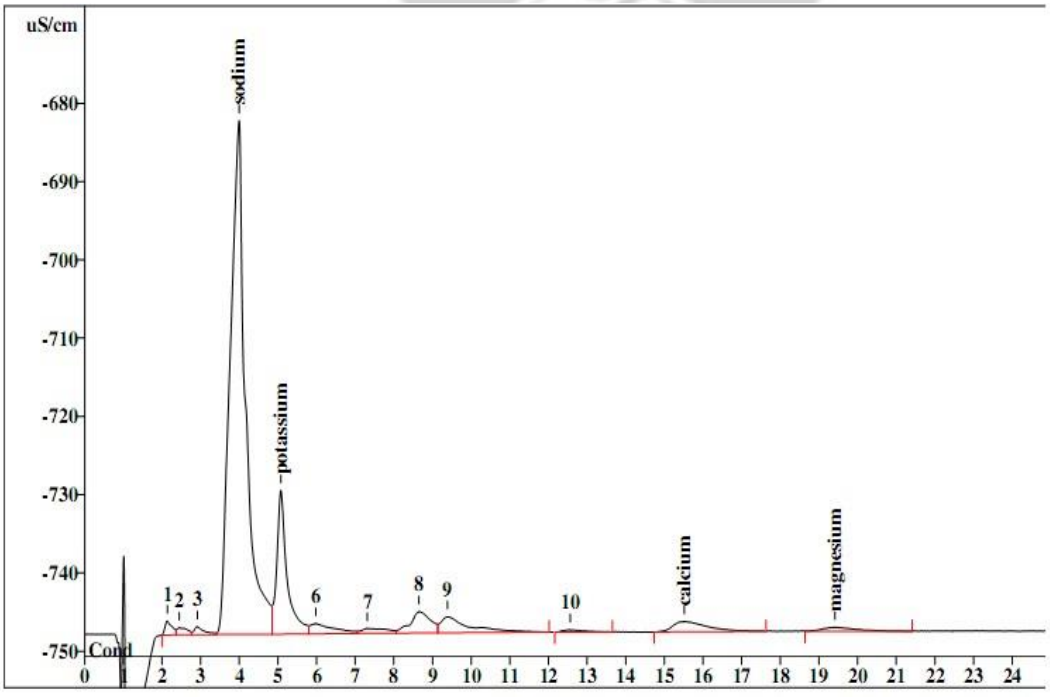

Figure 10 Cation in T2

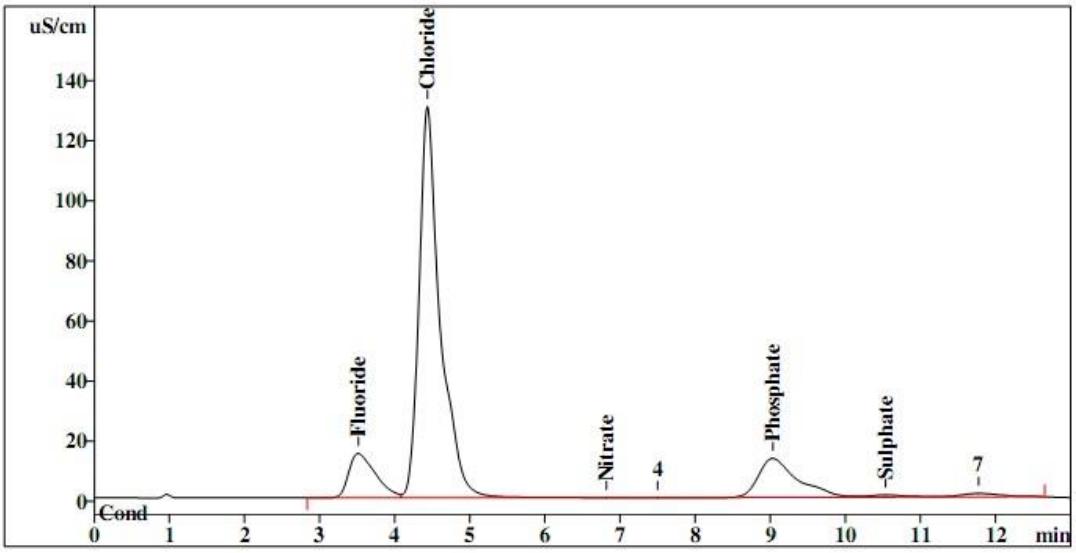

Figure 11 Anion in T3

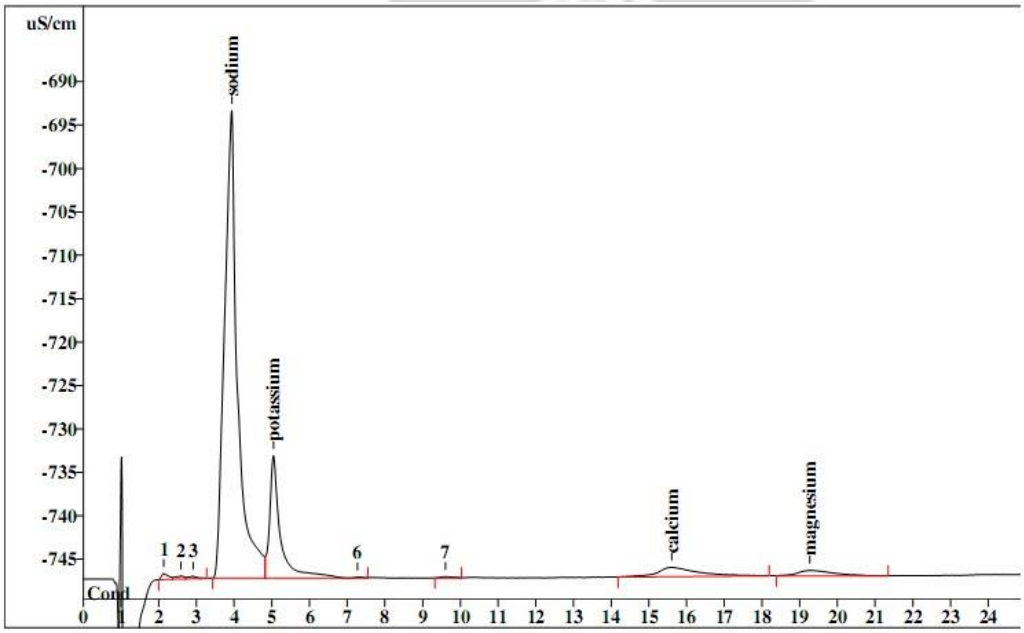

Figure 12 Cation in T3

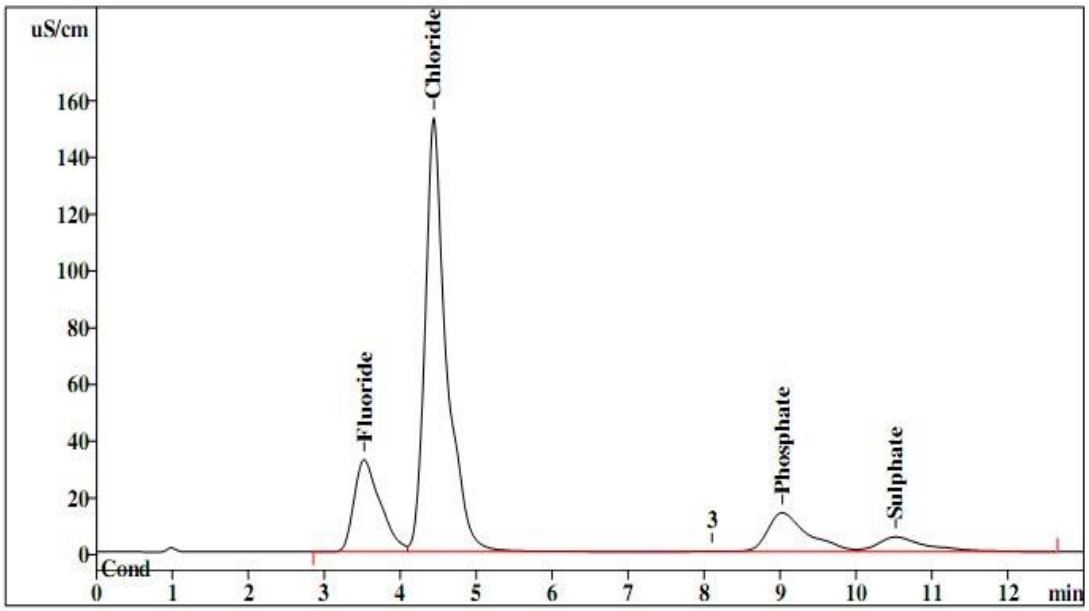

Figure 13 Anion in T4

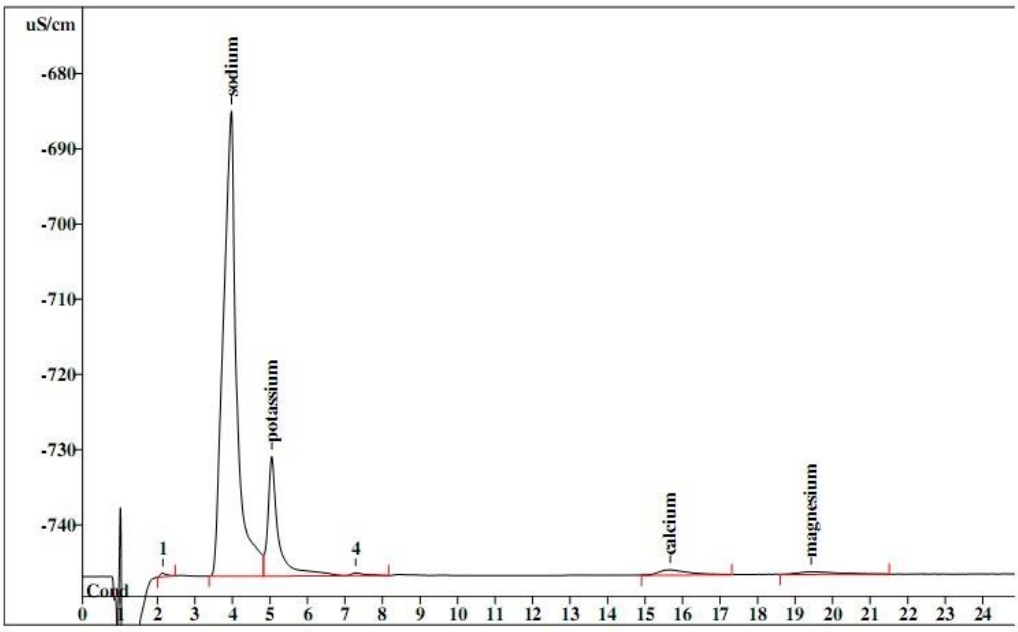

Figure 14 Cation in T4

7.13 Amino acids meat samples

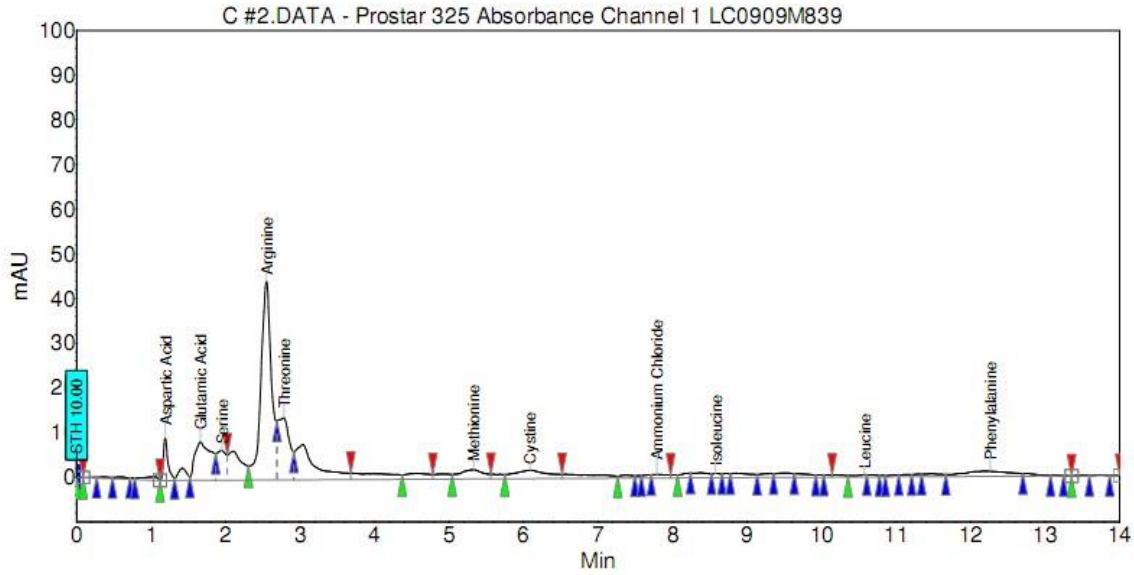

Figure 15 amino acids in catfish

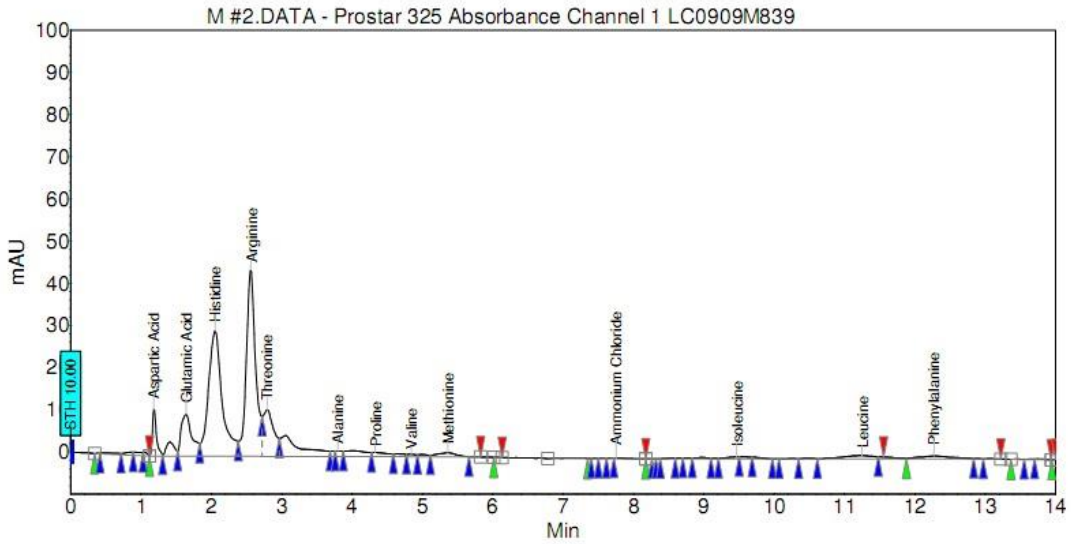

Figure 16 Amino acid in mackerel

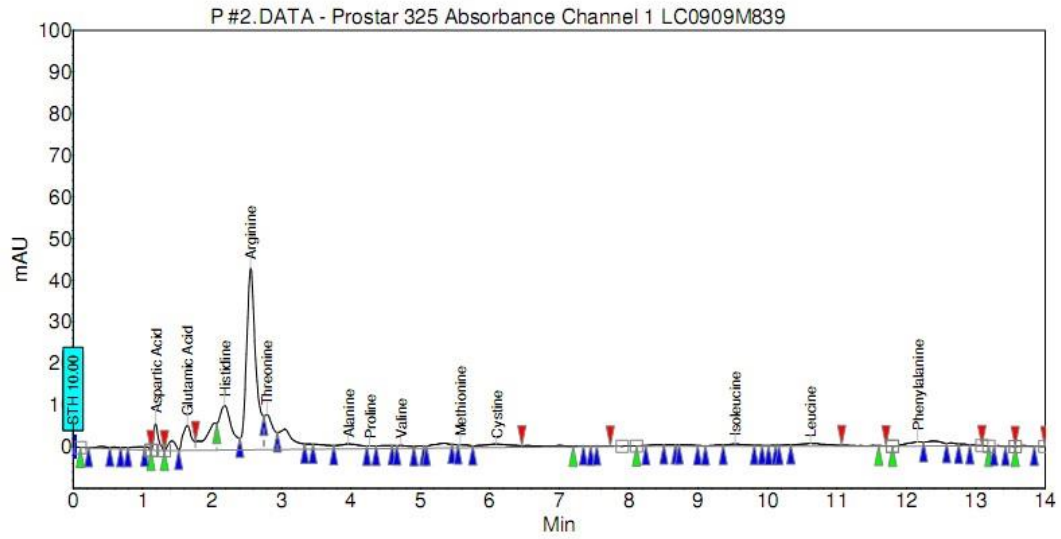

Figure 17 Amino acid in pork

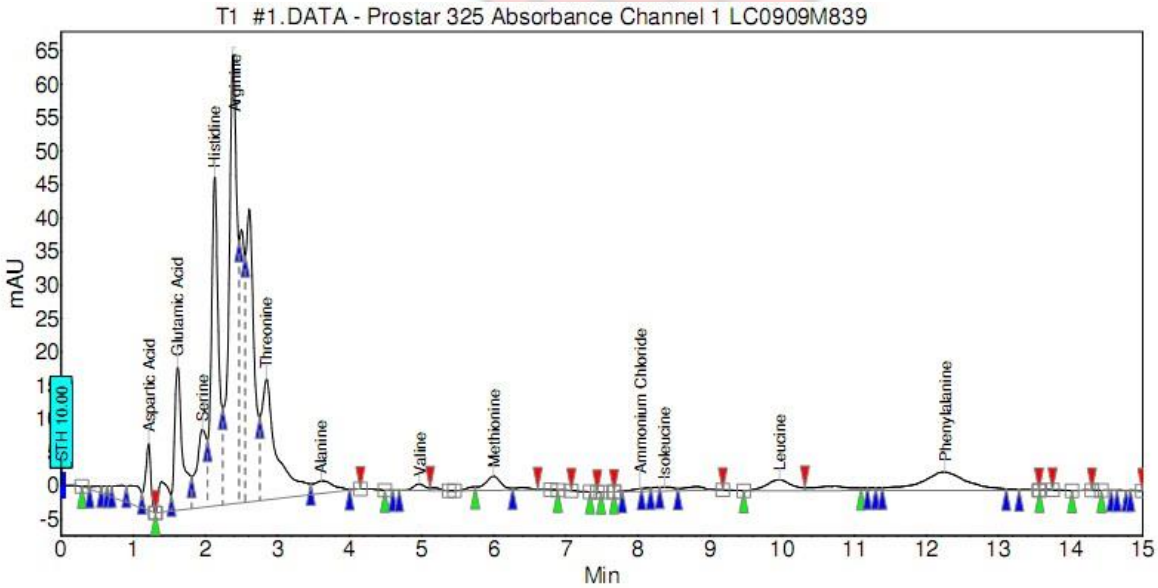

Figure 18 Amino acid in T1

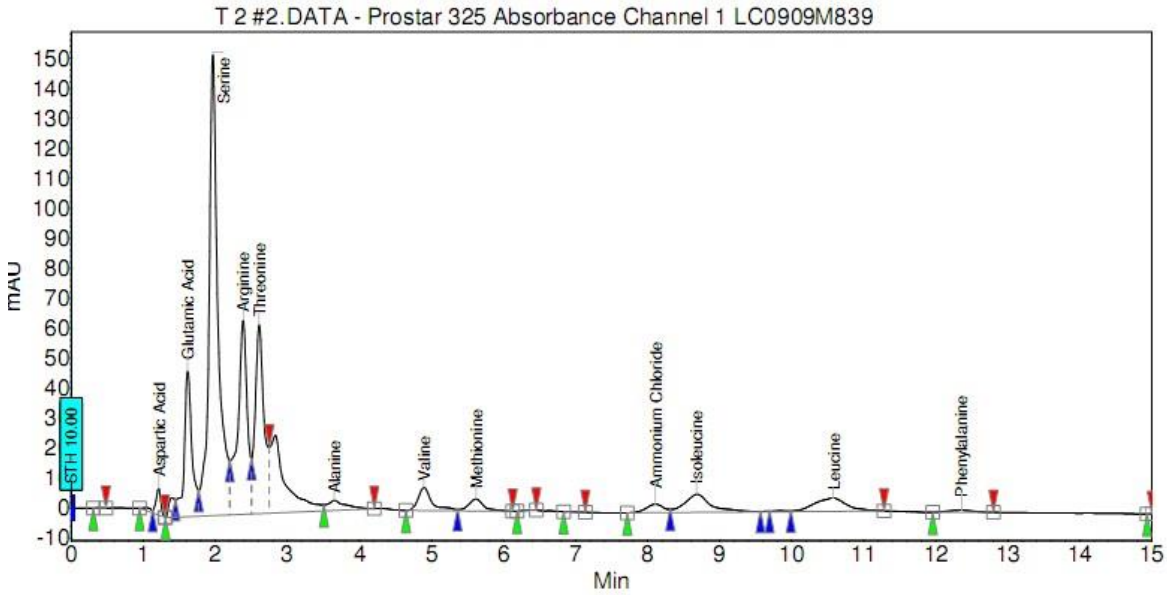

Figure 19 Amino acid in T2

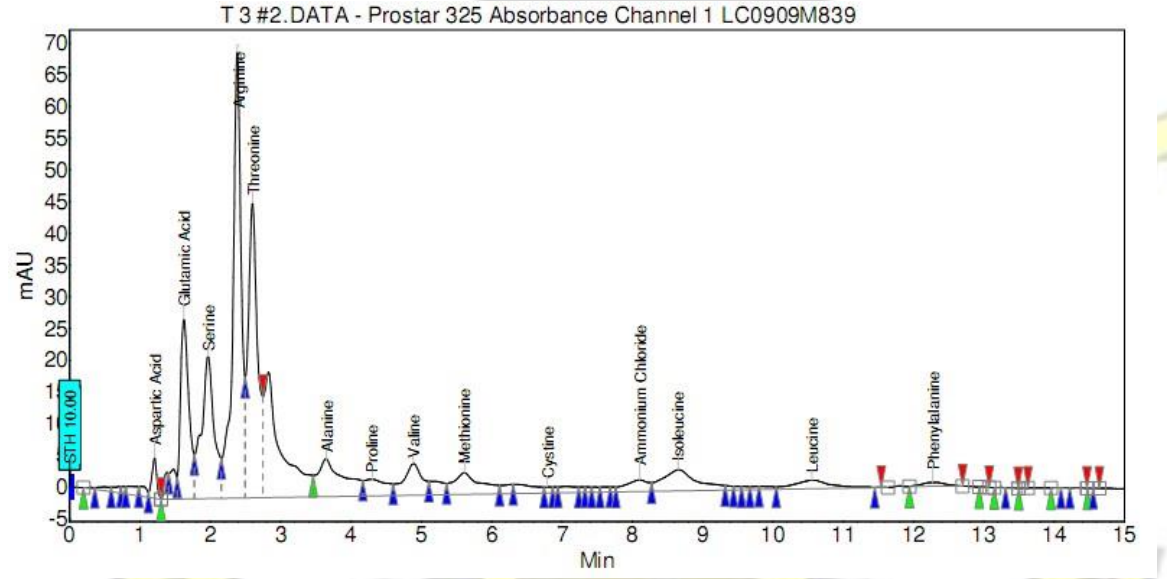

Figure 20 Amino acid in T3

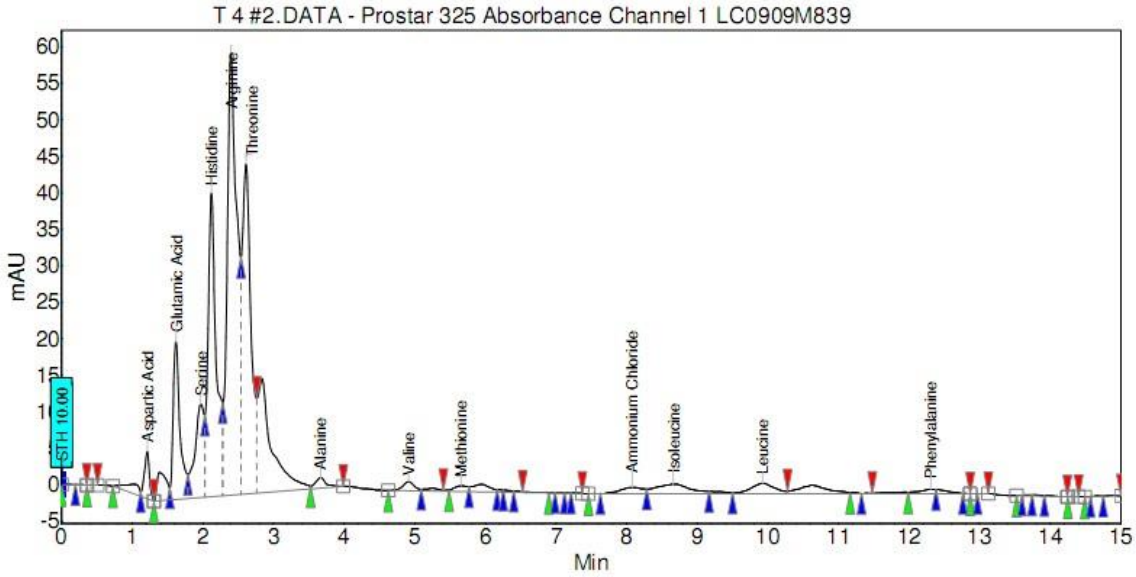

Figure 21 Amino acid in T4