THE PROXIMATE COMPOSITION, PHYSICAL, CHEMICAL AND MICROBIOLOGICAL PROFILE OF TILAPIA AROUND MINNA METROPOLIS

BY

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTERS OF TECHNOLOGY

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DECLARATION

I declare that this thesis titled "The proximate composition, physical, chemical and microbiological profile of Tilapia around Minna Metropolis" was carried out by me in the Department of Water Resources, Aquaculture and Fisheries Technology. The information derived from the literature has been duly acknowledged in the references. The work has not been presented for award of any degree at any university.

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28/2/11

Signature & DATE

CERTIFICATION

This thesis titled: "The proximate composition, physical ,chemical and microbiological profile of Tilapia around Minna Metropolis" by: Faremi Victor Akinwumi (M.tech/ SAAT/2007/1642) meets the regulations governing the award of the Degree of M.tech of the Federal University of Technology, Minna and is approved for its contribution to scientific

Knowledge and literary presentation.

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DEDICATION

Dedicated to the Glory of JEHOVAH NISSI, My all sufficient GOD and to my loving wife and children for their sacrifice of love.

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To the heavenly king of glory, I express my unalloyed joy for making what seems an impossible task a reality. I wish to express my profound gratitude to my major supervisor, DR J.O Oyero, for his advice and effectiveness in supervising this project to a successful completion and my gratitude and appreciation to Dr. S. B. Oyeleke of Department of Microbiology for his immense contribution to the completion of this project.

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I wish us all God's guidance, protection and abundant blessings as we all journey along the course of this temporal world.

ABSTRACT

An investigation was carried out on the application of HACCP on Tilapia species utilization in and around Minna metropolis in order to identify potential hazards and critical control points associated with the harvest and distribution network of fresh fish brought from different sources to the fish markets for sale to the final consumers. Fresh Tilapia fish samples were obtained from four (4) different locations within the period of May to July 2009 and these were analysed for potential physical, chemical and microbial hazards. Twelve species of microbes were identified from 64 samples of fish analysed. This study revealed the presence of physical hazards such as pieces of woods on the body, cut/abrasion and presence of Cu^{2+}, Pb^{2+} Fe^{3+} and Mn^{2+}. The results obtained showed a significant level ($P < 0.05$) of the effect of location on the bacterial load in the case of the first and third sets of samples while the second and fourth sets of samples taken showed no significant difference. The results also showed a significant difference on the effect of location on the presence of chemical hazards.

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CHAPTER ONE

1.0 INTRODUCTION

The Hazard Analysis and Critical Control Point (HACCP) system, is a science based system which identifies specific hazards and measures for their control to ensure the safety of food. HACCP is a tool to assess hazards and establish control systems that focus on prevention rather than relying mainly on end-product testing. Any HACCP system is capable of accommodating change, such as advances in equipment design, processing procedures or technological developments.

HACCP can be applied throughout the food chain from primary production to final consumption and its implementation should be guided by scientific evidence of risks to human health, as well as enhancing food safety, implementation of HACCP can provide other significant benefits. In addition, the application of HACCP systems can aid inspection by regulatory authorities and promote international trade by increasing confidence in food safety.

HACCP is a management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement and handling, to manufacturing, distribution and consumption of the finished product.

HACCP is designed for use in all segments of the food industry from growing, harvesting, processing, manufacturing, distributing and merchandising to preparing food for consumption. Food safety systems based on the HACCP principles have been successfully applied in food processing plants, retail food stores, and food service operations.

1.1 Classification of Hazards

Aquatic animals can be exposed to a range of hazards from the water to the table. Some of these hazards are natural to the aquatic environment; others are introduced by humans. The hazards can involve bacteria, virus, parasites, natural toxins, and chemical contaminants.

Hazard is defined as a biological, chemical or physical agent in, or condition of food with the potential to cause an adverse health effect. Hazards can thus be classified into three major categories: namely biological, chemical and physical.

Traditionally, industry and regulators have depended on spot-checks of manufacturing conditions and random sampling of final products to ensure safe food. This approach however tends to be reactive, rather than proactive and preventive, and can be less efficient, hence the need for application of HACCP procedures, which focuses on preventing hazards that could cause food-borne illness by applying science-based controls, from raw material to finished products. New challenges to food supply have prompted the food regulating agencies world-wide to consider adopting a HACCP-based food safety system on a wider basis. One of the most important challenges is the increasing number of new food pathogens. For example, between 1973 and 1988, bacteria not previously recognized as important causes of food-borne illness – such as *Escherichia coli* and *Salmonella enteritidis* – became more widespread.

There also is increasing public health concern about chemical contamination of food; for example, the effect of lead in food on the number of food industry and diversity in the amount of domestic food manufactured and the number and kinds of food imported. At the same time, federal, state and local agencies have the same limited level of resources to ensure food safety.

(FAO, 2004) reported that HACCP offers a number of advantages over the traditional system. Most importantly, HACCP;

- ❖ focuses on identifying and preventing hazards from contaminating food
- ❖ is based on sound science.

- ❖ permits more efficient and effective government oversight, primarily because the record keeping allows investigators to see how well a firm is complying with safety laws over a period rather than how well it is doing on any given day.
- ❖ places responsibility for ensuring food safety appropriately on the food manufacturer or distributor.
- ❖ helps food companies compete effectively in the international market

reduces barriers to international trade.

1.1.1 Biological Hazards

Biological hazards include bacterial, viral, fungi and parasitic organisms and also bacterial poisoning through the ingestion of natural toxins. These play a very large role in the incidence of food-borne diseases.

1.1.2 Chemical Hazards

Chemical hazards include chemical contaminants that are herbicides, pesticides and other chemicals such as lead, mercury, cadmium etc and also radioactive fallouts from nuclear tests and plant. All food products are made up of chemicals and all chemicals can become toxic at some dosage level. However certain hazardous chemicals are not allowed in food and others have allowable limits established.

1.1.3 Physical Hazards

These are often described as extraneous matter or foreign objects. They also include any physical matter not normally found in food, which may cause illness (including psychological trauma) or injury to an individual The most often reported complaint concerning physical hazards is that foreign objects provide tangible evidence of hazardous product deficiency.

HACCP is of great relevance and importance to fish quality in Niger state when the state at which the fish gets to the final consumer and the various stages of handling the fish undergoes predisposes the fish to spoilage and microbial infestation. HACCP has not been accorded the proper priority it deserves in Nigeria, bearing in mind the quality of fish made available to the consuming public.

1.2 Aims and Objectives

The aims and objectives of this study include the following:

(1) To identify potential and actual hazards to fish safety at the point of sales to final consumer.

(2) To analyze the samples to identify the actual microbes and heavy metals present in the fish put for sale at the fish market.

1.2.1 Specific Objectives

(i) To determine the proximate composition of the fish.

(ii) To identify physical, chemical and microbiological hazards of Tilapia around Minna.

(iii) To identify and characterized bacteria load in Tilapia around Minna.

1.3 Scope of Study

The scope of this research covers the study and investigation into the state of fish sold to the consumers in order to determine the suitability of the food for human consumption and also determine the hazards present in the fish consumed.

The extent of work is to investigate potential biological hazards and chemical hazards through microbial analysis, heavy metals assay, and physical inspection of samples.

1.4 Limitation of Study

The work is limited to Minna metropolis and surrounding landing sites such as Zumba fish market Shiroro, and Tagwai dam.

1.5 Justification of the Study

The occurrence of food borne diseases as a result of fish consumption by people in Nigeria and Niger state in particular has led to the need for investigation into the causes of these diseases.

There is also an increasing public health concern about chemical contamination of fish; for example effect of heavy metals in fish from polluted water sources and contamination arising from poor handling practices.

With all these problems there is therefore, the need to investigate the causes of this potential and real hazards.

1.6 Null Hypotheses

1. The proximate composition of the fresh fish sold in Minna fish markets did not differ significantly.
2. There is no significant effect of post harvest handling on Tilapia fish sold at Minna Market.
3. Tilapia sold at Minna Markets are not contaminated with microorganism and chemical pollutants.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Global view of food safety and Risk assessment in the fish industry

The emerging world trading system is committed to transparent rules relating to food safety and quality based on the principle of equivalence and a scientific approach. This is particularly important for fish and fishery products, which today are more internationally traded than any other food product. Whereas the concept of risk and food safety has been around for sometime, it was the agreement on the application of sanitary and phytosanitary measures (SPS) of the World Trade Organization (WTO) which came into effect in 1995 and set the stage for a risk approach to food control measure (FDA,1995). It states that safety and quality rules should where possible, reflect international standards such as those of codex Alimentarius, but different national standards can be applied as long as they are scientifically based using risk assessment.

The risk approach to food safety embraces the fact that whereas carefully designed preventive systems, such as HACCP, can produce safe foods, complete safety cannot always be guaranteed at all times for all people. Therefore communicating the risk associated with consumptions of different foods becomes of prime importance. The codex Alimentarius Commission (CAC) has identified microbiological risk assessment for foods as a priority.

The globalization of food trade and increasing problems worldwide with emerging and re-emerging of food borne disease have increased the risk of cross-border transmission of infectious agents. Because of the global nature of food production, manufacturing and marketing, infectious agents can be disseminated from the original point of processing and packaging to location thousands of kilometers away. It is therefore of paramount importance to understand how infectious agents enter and spread through the food chain in order to prevent or minimize exposure of the customer to such agents. This underscores the need to estimate the risk that food borne pathogens pose to human health in an international context and to identify possible interventions to reduce or eliminate these risks.

Food safety in the late 20th century and beyond requires enhanced levels of international cooperation in setting standards and regulations. Food safety measures are not uniform around the world and such differences can lead to trade disagreements among countries. This is particularly true if microbiological requirements are not justified scientifically.

2.2 History of HACCP

The HACCP concept had its origin in the USA and stands for "Hazard Analysis Critical Control Point".

New challenges to the U.S food supply have prompted FDA to consider adopting a HACCP-based food safety system on a wider basis. One of the most important challenges is the increasing number of new food pathogens. For example between 1973 and 1988, bacteria not previously recognized as important causes of food-borne illness such as *Escherichia coli* 0157:47 and *Salmonella enteritis* became more wide-spread. There also is increasing public health concern about chemical contamination of food: for example, the effects of lead in food on the nervous system. Another important factor is that the size of the food industry and the diversity of products and processes have grown tremendously in the amount of domestic food manufactured and the number and kinds of foods imported. At the same time, FDA and state and local agencies have the same limited level of resources to ensure food safety. The need for HACCP in the United States, particularly in the seafood and juice industries, is further fueled by the growing trend in international trade for worldwide equivalence of food products and the Codex Alimentarius Commission's adoption of HACCP as the international standard for food safety.

2.3 The HACCP Concept

This HACCP concept has to be developed for all products of every factory. The five basic ideas of HACCP – concept are:

1) Conduct a hazard analysis

2) Determine the critical points (CPs) which might be of hazard in the production of the food.

3) Determine the CPs which may be CCPs being of high importance to the safety of the food and which may be controlled safely using simple checks named "Controlling".

4) Define a control system of the critical points, using tests which can be carried out during production in order to interfere in case of wrong production. "monitoring".

Introduce documentation in order of every happening. Define corrections to be made incase of critical points being out of control.

5) Define the way of verification to confirm that the HACCP- system works. "Verification".

2.4 HACCP Principles

HACCP is a systematic approach to the identification, evaluation, and control of food safety hazards based on the following seven principles.(NACMCF,1997)

i. Analyze Hazards

Potential hazards associated with a food and measures to control, those hazards are identified. The hazards could be biological, such as a microbe, chemical, such as a toxin; or physical, such as ground glass or metal fragments.

ii. Identify Critical Control Points

These are points in a food production – from its raw state through processing and shipping to consumption by the consumer – at which the potential hazard can be controlled or eliminated. Examples are cooking, cooling, and packaging.

iii. Establish preventive measures with critical limits for esach control point

For a cooked food, for example, this might include setting the minimum cooking temperature and time required to ensure the elimination of any harmful microbes.

iv. Establish procedures to monitor the critical control points

Such procedures might include determining how and by whom coking time and temperature should be monitored

v. Establish corrective actions to be taken when monitoring shows that a critical limit has not been met

For example, reprocessing or disposing of food if the minimum cooking temperature is not met.

vi. **Establish procedures to verify that the system is working properly**

For example, testing time and temperature recording devices to verify that a cooking unit is working properly.

vii. **Establish effective record keeping to document the HACCP system**

This would include records of hazards and their control methods, the monitoring of safety requirements and action taken to correct potentials problems. Each of these principles must be backed by sound scientific knowledge; for example, published microbiological studies on time and temperature factors for controlling food borne pathogens.

2.5 Need, Benefits and Cost of HACCP

HACCP offers a number of advantages over the current system of food safety regulation. Most importantly, HACGP:

- Focuses on identifying and preventing hazards from contaminating food.
- Permits more efficient and effective government oversight, primarily because the recordkeeping allows investigators to see how well a firm is complying with food safety laws over a period rather than how well it is doing on any given day.
- Places responsibility for ensuring food safety appropriately on the food manufacturer or distributor.
- Helps food companies compete more effectively in the world market.
- Reduces barriers to international trade.

BENEFITS

To the Company (Producer)

- Production of safer food – lower business risk
- Improved/maintained reputation
- Compliance with legislation
- Staff have clearer ideas of food safety requirements and practices.

- Demonstrates company commitment to food safety
- Better staff organization/use of time.
- Long-term reduction in wastage (in the short-term wastage costs may go up due to corrective actions, requiring disposal of food as a result of failure to control CCPs properly).
- Less likely to receive customer complaints
- Possible increase in market access.

To Customers:

- Less risk of illness.
- Improve quality of life.
- Greater confidence in food.

To Government

- Facilitating food safety inspections/more efficient food control.
- Improved public health/reduced health care costs.
- Facilitates international trade.
-

2.6 Barriers to implementing HACCP

Introducing HACCP or revising an existing HACCP scheme requires care in preparation and planning because introducing HACCP into a company for the first time is likely to involve a major change to the way things are managed. How successful this introduction will be is dependent upon the skills of the HACCP project coordinator or team leader. The person requires process and technical skills – process here refers to managerial and interpersonal skills such as ability to head and manage. Technical skills relate to food safety and product knowledge as well as scheduling, budgeting, etc.

The introduction of HACCP, in spite of any legislative requirements may still be faced with some resistance or antagonism just because it is different; hence the implementation of HACCP is sometimes faced with the following barriers.

2.6.1 Lack of finance and resources

Lack of finance and resources is especially more pronounced in small business. But recent studies according to Dillion, and Griffith (2001) suggest costs of HACCP whilst proportionally greater for small businesses are affordable – time may often be more of a problem than direct cash costs.

2.6.2 Lack of Government Commitment

There is presently low level of government commitment but this is likely to become less of a problem in the future because of the current global enlightenment of the citizenry. Increasingly HACCP is recognized as the best way to improve food safety. Within the European countries, HACCP principles are incorporated into EC directive 93/43. Codex recommendations advocate use of HACCP plus international agreed principles, greater government pressure to include food service establishments especially where tourism is important.

2.6.3 Lack of customer and business demand

There has been reports from many countries of tourists (up to 50% in some countries) suffering gastro intestinal infections with greater liability on the travel operators to use "safe hotels", and this has also led to greater demand on suppliers by retailers and manufacturers.

2.6.4 Human resource constraints

Lack of skilled workforce. More HACCP courses to an agreed training standard will help to correct this.

2.6.5 Lack of technical support

There is need for more books, consultants, training packages and information on hazards and risk. There is also need for government guides to implementation.

2.6.6 Inadequate support and facilities

In order to fully implement HACCP in most countries particularly developing countries, it will require the phasing out of older poorly designed factories for economic reasons.

2.6.7 Inadequate communications

Inadequate communications is still a major problem for smaller companies/food service and developing countries.

2.6.8 Staff resistance

Staff resistance would come as a result of a combination of the following reasons

i. **Personality Problems** – Personality clash with person implementing HACCP.

ii. **Self Interest** – Perceptions that the new way of doing things may result in loss of status to an individual

iii. **Lack of knowledge** – Don't know what HACCP is all about or why it is needed.

iv. **Psychological Reasons** – Fear of the unknown mystique of HACCP, fear of being unable to do HACCP.

v. **Cultural Reason** – Because HACCP is different from the old ways of doing things.

vi. **Emotional Reason** – Don't accept the need, can't be bothered, uncertainty.

vii. **Method of Introducing HACCP** – Indifferent or resentment caused by lack of communication skills during the introduction, lack of staff involvement.

viii. **Staff Time** – Time is an important factor in both designing and maintaining a HACCP plan.

2.7 Developments in food safety and quality systems

Food quality including safety is a major concern facing the food industry today. A number of surveys have shown that consumer awareness about quality of their food is increasing. The extensive coverage in the daily press of food safety issues concerns about genetically modified foods, use of growth promoters, existence of pesticide and doxinresidues in food, the salmonella problem, transfer between micro organisms of resistance to community used antibiotics add to consumers fear and unease about what they eat. Recent events around the Globe, such as the Bird flu (avian influence) and swine flu are very good examples of such awareness.

The situation is further complicated by the fact that many consumers suffer from a serious lack of knowledge on simple food safety issues. Thus less than one percent of U.S and Canadian consumers met minimum criteria for acceptable safety practices in a North America audit of food preparation behaviour in which 106 consumers agreed to be watched while preparing food (Daniels, 1998), in a similar study, only 4.7% of UK consumers fully implemented appropriate food safety contra practices (Griffith *et al*, 1998). Furthermore, most consumers exhibit a general disbelief in the importance of good handling practices and a great resistance to effective protective

treatment such as chemical preservation or irradiation. As a consequence, there is an increasing demand for fresher or even raw food with enhanced natural flavours and products with less or no use of salt and other preservatives. A great number of socio-economic changes such as increased urbanization (crowding), migrations and population demographics are further contributing to the safety of foods. The population of highly susceptible person is expanding worldwide because of ageing, malnutrition, HIV infections and other underlying medical conditions with a weakened immune system. To meet those challenges, food manufacturing is becoming a highly complex business, particularly since raw material is sourced on a global scale and new processing technologies are used to produce a vast array of products. Much research is needed to evaluate new techniques and to consider food safety issues at all stages from production of raw materials to sale of final product.

Despite great efforts in research, food borne diseases continue to present a major problem of both health and economic significance. The cost of food-borne diseases is high. Although the full economic impact is not known, preliminary estimates in the United State in 1994 placed the cost between US$ 10-83billion (FDA, 1997) some of this huge cost is borne by the food-producing company-and loss of consumer confidence may even cause bankruptcy but the great majority is borne by the government. It has become overwhelmingly clear that all countries need an adequate food control programme to ensure a safe food supply to protect and promote the health of the consumer.

Fish and fishery products are in the forefront of foods safety and quality improvement because they are among the most internationally traded food commodities. In 2001, fish trade amounted to US$54,000million, of which appropriately 50percent originated in developing countries (FAO 2004). Since 1994, more and more fish has been used for direct human consumption rather than for other purposes. Of the products used for human consumption; fresh fish showed significant growth during the 1990s and almost 50% of fish used for human consumption is sold fresh(FAO,2004). This change has been accompanied by a decline in the use

of cured and canned fish. Also proportion sold as frozen fish is declining. This pattern has largely been driven by growth in consumption.

Fish has a significant capacity for processing and almost two thirds of the catch (in 1998) were used for further processing (FAO, 2004). A large fraction, approximately 30% of the fish used for human consumption was frozen, approximately 14% canned and approximately 12% cured. The remaining 45% was sold fresh(FAO, 2004).

Different regions of the world have very different eating habits with respect to seafood. Demersal fish such as cod are much preferred in Northern Europe and North America, and cephalopods are consumed in several Mediterranean and Asian countries, but to a much lesser extent in other regions.

The traditional approach to food safety assurance was based on applying codes of Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP) in food processing. Confirmation of safety and identification of potential problems were obtained by end – product testing. Inspectors checked for compliance with the codes and sampled the foods for laboratory analysis. In contrast, the HACCP system clearly identifies food safety problems and also where and how they can be controlled or prevented. To assure that these actions are executed regularly and consistently, they have to be described and people who are responsible for their execution have to be trained. A record keeping system has to be developed to provide documentation for all actions and measurements. Originally, HACCP was developed and used by the private food industry. The concept was used by the private food industry. The Pillsbury Company used the concept in the late 60s for the safety of food intended for the US space program. However, it took many years and endless discussions between regulatory agencies and the food industry on the value of end-product testing and microbiological standards for the food before the HACCP concept was generally accepted as the primary means to assure food safety (FAO, 2004).

Although the HACCP system both in EU and US is based on the same seven principles, there are some differences between the two systems. These differences are mainly related to the

prerequisite programmes, the way they are documented and verified, and the scope and content of the identification of hazards (FAO, 2004).

Until April 1995, acceptance of the work of codex by the member governments was voluntary. However, with the establishment of the World Trade Organization (WTO) in April 1995 the situation has changed. According to two of the Agreements of the WTO (the Agreement on sanitary and phytosanitary measures (SPS) and the Agreement on Technical Barrier to Trade (TBT), the work of codex is recognised as the reference for internationally food safety requirement. This implies that in the future member states of WTO cannot reject food, which meets codex recommendation and standards without providing justification based on risk assessment. Since the application of HACCP has become the international reference system for food safety assurance (FAO, 2004).

2.8 Risk Assessment

The use of risk assessment has gained steadily in importance and recognition as the scientifically-based approach for the development of food safety and quality standards. The emphasis on risk comes from the logical extension of the Hazards Analysis Critical Control Point (HACCP) revolution that swept the industry in the 1980s and 1990s. HACCP principle 1 states that a hazard analysis must be done. First those hazards that are likely to occur are identified, and then an assessment is made of the severity of each hazard, followed by and evaluation of its likelihood to occur. These two factors (severity and likelihood) tell us about risk.(NACMCF,1997).

Risk analysis is a process of three components:

- risk assessment
- risk management
- risk communicating

Risk assessment is a scientifically based process consisting of the following steps:

- hazard identification
- hazard characterization

- exposure assessment
- risk characterization

The aim of risk assessment is to estimate the level of illness that may be expected in our target population from a product or group of products.

The information flow for the four components in a risk assessment is shown below:

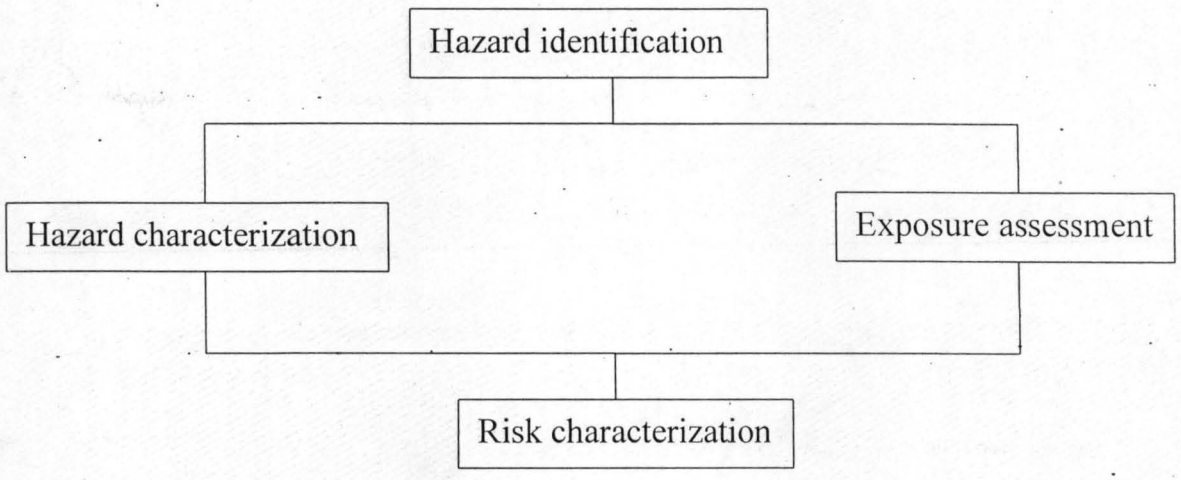


Fig. 2.1 Component of Risk Assessment

Hazard Identification

The identification of biological, chemical and physical agents capable of causing adverse health effects and that may be present in a particular food or group of foods.

This is the first stage in risk assessment and is a screening process to make certain that the hazard really does not exist in this particular product. For example, *Clostridium botulinum* is readily identified as a hazard in canned, smoked and vacuum-parked seafoods, but is unlikely to be a hazard for any other seafood product. So hazard identification is a primary screen that allows fish managers to eliminate products; pathogen pairs that are of no concern. (Summers, *et al.* 2001).

Hazard characterization

The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents that may be present in food. For the purpose of microbiological risk assessment the concerns relate to micro-organisms and/or their toxins.

There are two parts to hazard characterization:

- description of the effects of the hazard (micro-organism or toxin);
- the dose-response relationship (if it exists)

Exposure assessment

This is the qualitative and/or quantitative evaluation of the likely intake of biological, chemical and physical agents via food as well as exposures from other sources if relevant.

Risk characterization

This is the process of determining the qualitative and/or quantitative estimation, including attendant uncertainties of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment. When the risk characterization is done, it involves the integration of hazard characterization to provide an estimate of the risk.

2.9 Types of Risk Assessment

There are several types of risk assessment that fall under three broad categories:

(i) qualitative risk assessment

(ii) semi-quantitative risk assessment

(iii) quantitative risk assessment

All three categories provide useful information and the choice of assessment to employ will depend on the speed and complexity required from the assessments.

2.9.1 Qualitative risk assessment

These are the simplest and quickest to do, but they can be rather subjective, which reduces their value. Every HACCP plan contains simple qualitative risk assessments in the HACCP worksheet. For every hazard, an estimate of risk is made by inserting high, medium or low in answer to questions on the severity of the hazard and the likelihood of it occurring. A basic problem is that the three descriptors (high, medium, low) are often inadequate foe example, suppose the process step is retorting in fish canning and the hazard is *clostridium botulinum. Almost* everyone will

describe the severity of the hazard as high. But how likely is the hazard to occur? Most people will put low because billions of cans of fish are manufactured each year with no sign of the hazard. High severity and low likelihood – how would you link these to estimate risk? (NACMCF, 1998).

Type 1: Hazard control worksheet

Process Step	Hazard	What can go wrong	Severity of Hazard	Livelihood of occuring	Hazard control
	BIOLOGICAL				
	CHEMICAL				
	PHYSICAL				

Another type of qualitative risk assessment is shown below in which the risk estimate is a risk ranking high, low and medium.

Type 2: Qualitative risk ranking

Process Step	Product	Severity of Hazard	Livelihood of occuring	Exposure in diet	Linkage with epidemiology	Risk ranking

This assessment is based on factors which are linked with exposure assessment (likelihood of occurrence and exposure in the diet) plus one which is linked with hazard characterization (severity of hazard). If the hazard: product paring has some linkage with epidemiology (it has caused food poisonings), this serves to remind you that there is some probability that it will happen again.

Another qualitative scheme for categorizing risk from seafood has been developed by Huss, and Embarek (2000) who ascribe pluses to hazard, then rank risks as "high" (four or more pluses) or "low" (less than four pluses).

The scheme takes into account epidemiology (bad safety record) and then focuses on the process, searching for a Critical Control Point (CCP) for each hazard and assessing possibilities for growth and death of microbial hazards.

Type 3: Qualitative risk assessment based on the process

Risk criteria	Raw molluscan shell fish	Canned fish	Dried fish
Bad safety record	+	+	-
No CCP for the hazard	+	-	-
Possibility of contamination or	+	+	-
recontamination	+	-	-
Abusive handling possible	+	-	-
Growth of pathogen can occur	+	+	+
No terminal heating step	High	Low	No Risk
Risk category			

Source: after Huss, and Embarek (2000).

So, as shown in Type 3, molluscan shellfish, fish eaten raw, lightly – preserved fish and mildly heat – treated fish are considered "high" risk, while chilled/frozen fish and crustaceans, semi – preserved fish and heat – processed (canned) fish are considered "low" risk; dried and heavily salted fish are considered to have no risk.

2.9.2 - Semi – quantitative risk assessment

In qualitative risk assessment, we estimate risk according to subjective terms such as high, low or medium. In semi-quantitative risk assessment we obtain a numerical risk estimate based on a mixture of qualitative and quantitative data. To do this type of assessment you need much of the data that will be used in a full quantitative risk assessment.

Ross and Sumner (2002) developed a simple spreadsheet tool to describe the risk that emerges from pathogens in products manufactured by typical process (canning, chilling, cooking etc) Table 1 lists risk criteria needed for a semi-quantitative risk assessment. These are simple questions and they can be answered qualitatively in terms such as "high' and "low".

2.10 Prerequisite to HACCP

HACCP is a necessary but not sufficient condition for ensuring food supply chain safety. In other words HACCP cannot be effective when applied as an isolated system, it must therefore be supported by pre-requisite programs (CAC, 2001).

Each company is thus required to have its own required pre-requisite programs prior to the implementation of the HACCP system.

Hygiene standards and procedures usually described as Good Hygiene Practices (GHP) of Good Manufacturing Practices (GMP), have been in place for many years and constituted an essential tool in traditional food control. These concepts are still essential in a modern food control system by providing the basic environmental and operating conditions for production of safe food and thus being a requisite or foundation for HACCP in an overall food safety management programme (Figure 2.3) what is new is the concept of formalizing the pre-requite programme alongside HACCP and the legal requirement in some countries (USA) of documented monitoring of certain sanitation areas.

Good Manufacturing Practices (GMP) are those procedure for a particular manufacturing operation which practitioners of, and experts in that operation consider to be the best available using current knowledge.

There is no clear definition of the term Good Hygienic Practices (GHP). However, "food hygienic" has been defined by Codex (CAC, 2001) as "all conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain" and GHP can therefore basically cover the same ground and for the purpose of this write-up, the term GHP will mainly be used.

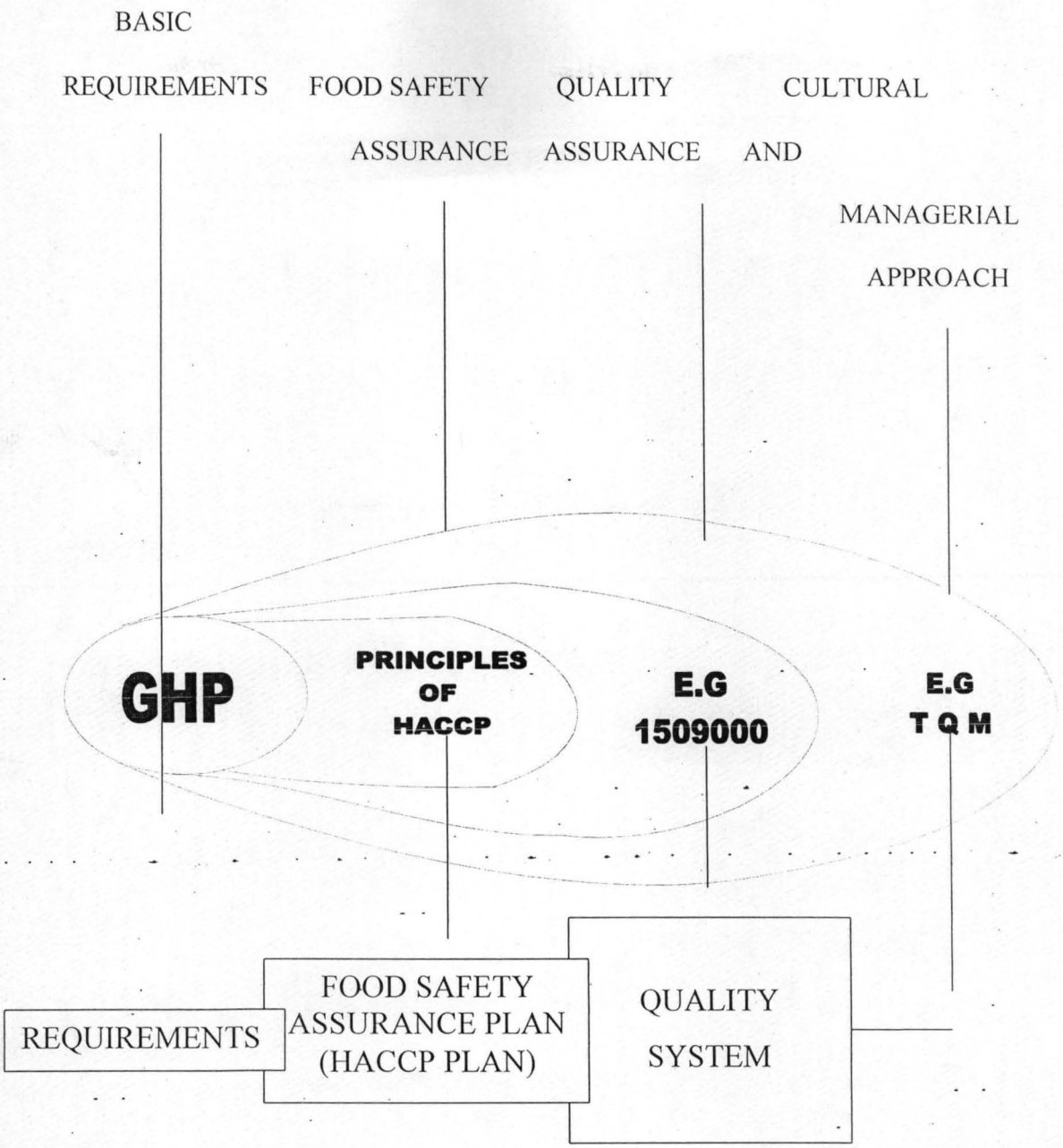


Fig. 2.2 Food safety and quality an integrated approach (Source (Jouve, 1998).

Various definitions of GHP or prerequisite programmes have been proposed by national and international organizations as shown:

Prerequisite Programme = Good Hygienic Practices (GHP)		
Prior to the application of HACCP to any sector of the food chain that sector should be operating accordance to Codex General Principles of Food Hygiene appropriate code codes of practices, and appropriate (CAC, 2001)	Practices and conditions needed prior to and during the implementation of HACCP and which are safety (WHO, 1999).	Procedures including GMP that address operational conditions providing the system (NACMCF,1998)

2.11 Applications of the HACCP Principles

Guidelines for the application of the HACCP system have been presented by CAC (1997). In these guidelines it is pointed out that, prior to prior to application of HACCP to any food operation, this sector should be operating on the basis of a prerequisite programme as outline in section 2.5.0 of the write-up. Furthermore, it is essential that top management is firmly committed to introduce the system. Many departments and different personnel from chiefs to the line operators will be involved and responsible for part of the system, and their full support and cooperation will be needed.

The Codex guidelines suggest that the introduction and application of the HACCP principles should follow a series of 12 steps in logic sequence as described below:

Step 1: Assemble the HACCP team

Introduction of a HACCP system is large food factories is a complex process and requires a multidisciplinary approach by a team of specialists. The microbiologist is of paramount importance, and must advice the team on all matters related to microbiology, safety and risks. He

must have an updated knowledge on these matter and also access to technical literature on the most recent developments in his field.

Another important member of the HACCP team is the processing specialist. He must advice on production procedures and constraints, prepare the initial process-flow diagram, advice on technological objectives at various points in the process and on technological limitations of equipment. Other technical specialist such as a food chemist a food engineer as well as packaging technologists, sales staff, training and personnel mangers can provide valuable information to the HACCP team and they should attend some of the meetings. (NACMCF, 1997)

Key members of the HACCP team (including the leader) must have an intimate knowledge of the HACCP system when the HACCP team is assembled, the scope of the HACCP plan should be identified, describing which segment of the food chain is involved and addressed in the work (NACMCF, 1997).

Step 2:Describe product

A full and detailed description of the final production must be drawing. The raw materials and ingredients used must be specified including the market name or Latin name of the fishery component. Details regarding hazards in the raw material will be included in the HACCP plan. All factors which influence safety such as composition, physical/chemical structure including water activity (aw) and pH must be described, and any microbiocidal/static treatment such as heating, freezing, bring and smoking must be specified as well as packaging type, storage conditions and methods of distribution. The normal shelf life under specified conditions should also be recorded as shown below (NACMCF, 1997).

Element of the Product description

1) Product Name
2) Raw material and ingredients used
3) Parameters influencing safety (9w, PH Salt% etc)
4) Processing
5) Packaging and Packaging materials

6) Storage conditions and shelf life

7) Conditions during distribution

8) Intended use and consumer

9) Labeling constructions

Step 3:Identify intended use and consumer

The HACCP team will need to identify the intended use and consumer of the product. The intended use should be based on expected use by the consumer. The use and preparation before use greatly influence the safety of product. Certain products may be contaminated or carry pathogenic organisms as a part of the natural flora. If the processing does not include the killing step, the only critical control point (CCP) which can render the product safe is adequate hear treatment during preparation.

The intended consumer may be the general public or a particular segment of the population such as infants or elderly. If the product is to be sold to hospitals or groups of th population with high susceptibility, more safety is required and critical limits need to be more strict.

Step 4:Construct flow diagram

The purpose of the flow diagram is to provide a clear simple description of all steps involved in the processing. Receiving storage steps for raw materials and ingredients should be included. Time and temperature conditions during processing should be mentioned whenever there is a holding step e.g. in holding vats, buffer tanks or other areas, where this could be a potential delay in processing.

Step 5:On-site conformation of flow diagram

The constructed flow diagram should be verified on-site for accuracy. The site should be inspected during all hours (night shifts, weekends) of operation to check for correctness and ensure that nothing crucial was overlooked.

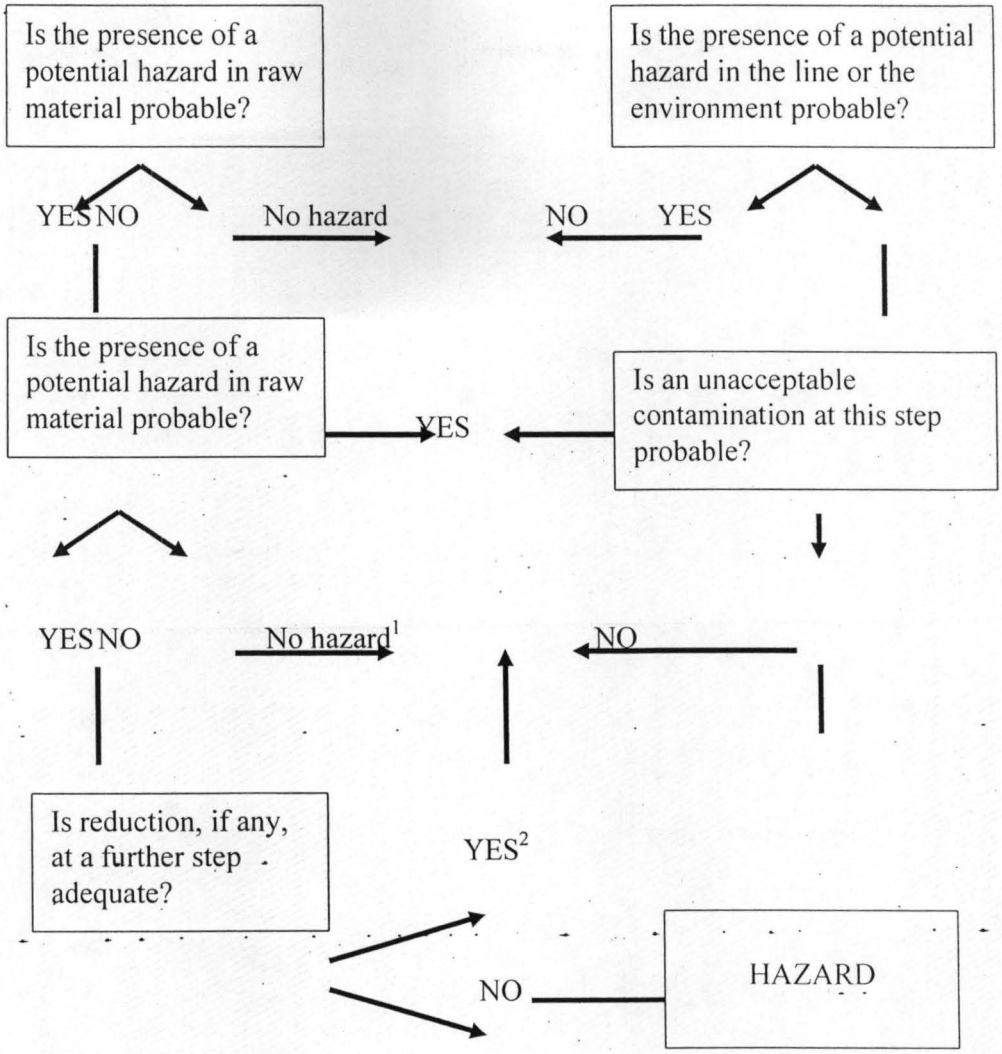
Step 6:List all potential hazards associated with each step in the operation, conduct hazard analysis and consider any measure to control identified hazards (principles 1)

- The hazards and associated control measures are identified,

- Needed modifications to a process or product is identified,
- Providing a basis for determining CCPs (Principle 2).

Examples of questions to be considered, when conducting a hazard analysis has been listed by NACMCF (1997) and includes the following; but the conditions covered by the prerequisite programme have been excluded from the list;

A decision tree with a number of questions can be used to determine if potential hazards are "real" as demonstrated in figure 2.3.



1. Not a hazard to be controlled at this step

2. Thus, reduction step becomes CCP

Figure 2-3-Hazard determination – Question to be answered for each potential hazard at each step (based on ILSI, 1997).

The questions in Figure 2.4 have to be asked at each step of the processing chain and all hazards must be considered. An element of risk assessment is involved in the evaluation of potential hazards. Only these hazards which are likely to occur and which will cause a reasonably serious adverse health effect are regarded as significant as shown in figure 2.4.s

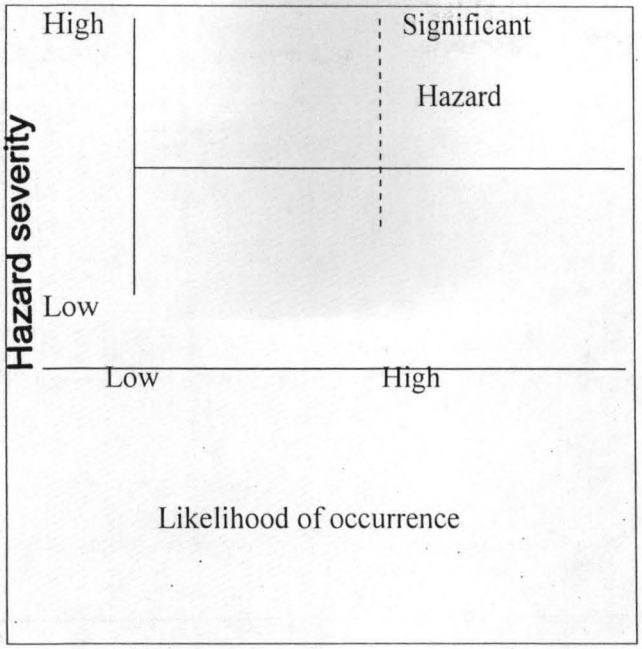


Figure 2.4. Determination of hazard significance

(after Mortimore and Wallance, 1998).

Thus, the basic procedures to use in conducting the hazard analysis are as follows:

i. Based on the product description and the flow diagram, all potential hazards associated with the product and at each processing step is determined and listed

ii. Make a hazard evaluation:

   a. Assess severity of health consequences if potential hazards are not controlled

   b. Determine likelihood of occurrence of potential hazards if not properly controlled

   c. Using information above, determine if this potential hazard is to be addressed in the HACCP plan

   d. Describe control measures.

Control measure(s) is (are) any factor or activity, which can be used to prevent eliminate or reduce safety hazard to an acceptance level. More than one control measure may be reuired to control a hazard.

Upon completion of the hazard analysis, the hazards associated with each step in the production should be listed along with any measure(s) that is (are) used to control the hazards. A "hazard analysis worksheet" can be used to organize and document the considerations in identifying food safety hazard. An example of a hazard analysis worksheet is shown in Appendix 1.

Step 7: Determine the critical control point (CCPs) (Principle[2])

Complete and accurate identification of all the CCPs is fundamental to controlling food safety hazards. To facilitate this is identification, the use of a CCP decision tree can be of great help. Examples of decision tress are found in NACMCF (1997) CAC (1997) and in the ILSI (1997) document.

Critical Control Point (CCP) is a step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level (CAC, 2001) figure 2.6 illustrates an example of decision tree.

The first two questions in figure 2.6 deal with the raw material. It is important to note that if an identified hazard is eliminated or reduced at a later process step or by normal consumer use, the raw material is not a CCP. Question 3 deals with formulation or composition of the product. Question 4 asks, if contamination, recontamination or even multiplication of pathogens can take place at this step. If the answer is "No" question 6 thus has to be answered, but if the and answer is "Yes", the and answer to question 5 will decide whether this step is a CCP or not.

Only points where truly significant hazards can be controlled should be designated CCPs. A tendency exists to control too much and to designate too many CCPs. This should be avoided as it will create confusion and divert attention from the true CCP.

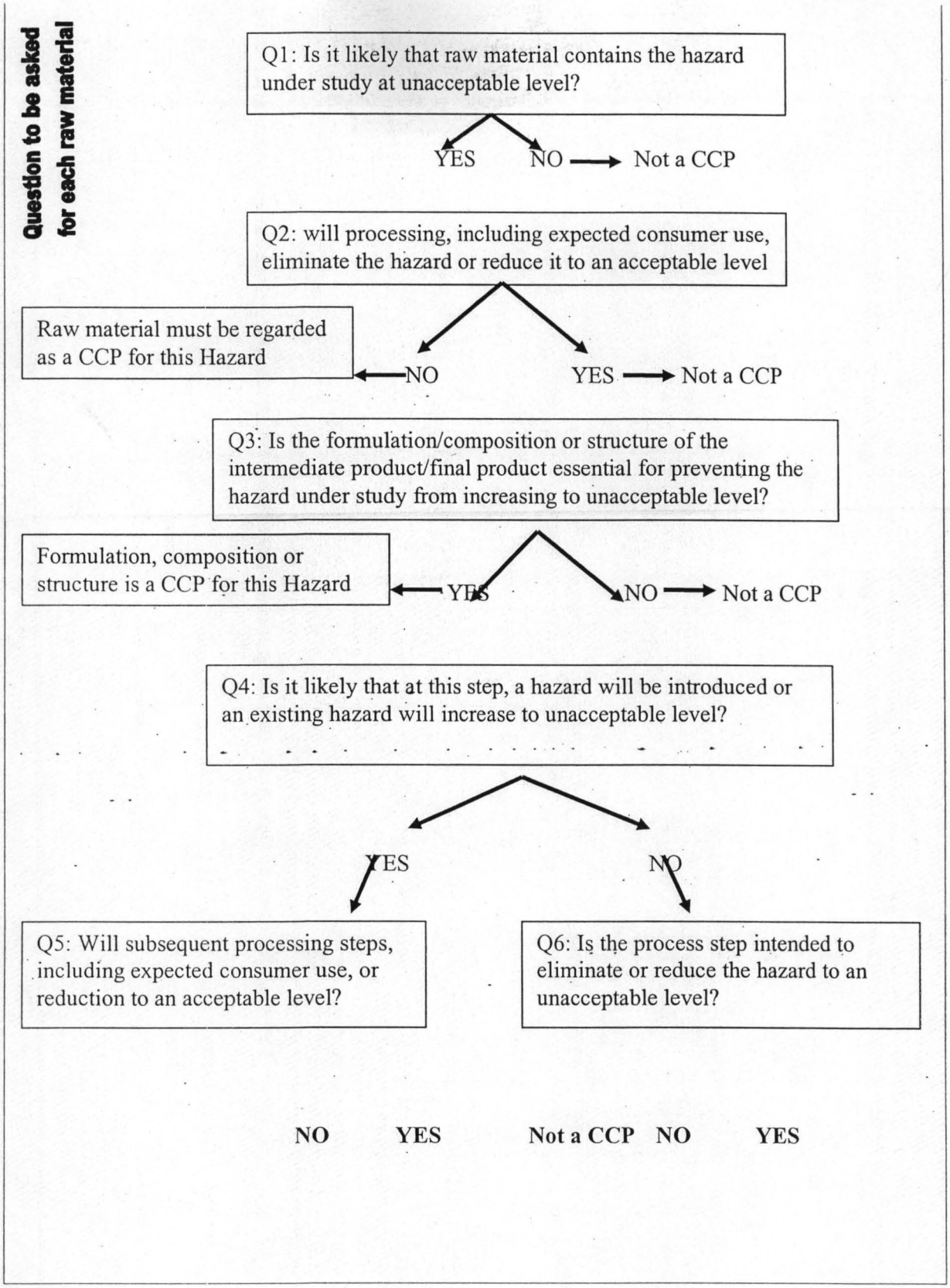


Fig 2.5 Critical control point decision tree (ILSI, 1997)

Step 8: Establish critical limits (Principles 3)

The third HACCP principles deals with establishing one or more maximum or minimum critical limits that must be controlled at each CCP.

Critical limit is a criterion, which separates acceptability from unacceptability (CAC, 2001). All critical limits should be scientifically based and refer to factors such as time (temperature conditions, moisture level, water activity aw) pH, titratable acidity, salt concentration, available chlorine, preservatives, Organoleptic or sensory quality.

Authoritative critical limit information is available from sources such as the "Fish and Fisheries Products Hazards and Control Guide" (FDA, 1998) or may be found in scientific publications or obtained from regulatory agencies. When critical limits have been established, they should be entered on the "HACCP PLAN FORM" An example of a HACCP plan form is shown in Appendix 2

Step 9: Establish monitoring procedures (Principle 4)

Monitoring of CCP serves three purposes (NACMCF, 1997):

- to determine if there is a loss of control and a deviation occurs at a CCP. Appropriate action must then be taken.
- monitoring keeps check on the operation and provides information whether there is a trend towards loss of control and action can be taken to bring the process back into control before a deviation occur.
- provides written documentation for use in verification and audit. All records must be signed.

Monitoring is the act of conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP is under control (CAC, 2001).

To be effective, all monitoring must be done rapidly and results must be evaluated by a designated person with knowledge and authority to carryout corrective actions. Typically, monitoring methods are:

- time/temperature recording

- pH and aw measurements
- sensory quality.

Thus, in planning the monitoring procedures there are typically four questions to be answered (CAC, 2001).

Planning monitoring procedures

- ***what***– usually a measure or observation
- ***how*** -by observation and/or use of instruments
- ***when*** - (frequency) – continuous or intermittent – but in

real time.

- ***Who*** - someone who is qualified and with authority.

As already stated, the main purpose of monitoring is to determine if there is loss of control or derivation.

Deviation is failure to meet a critical limit (CAC, 2001)

An example of a process being in control and out of control (deviation) has been illustrated by Motarjemi and Van schothorst (1999) as shown is figure 2.8

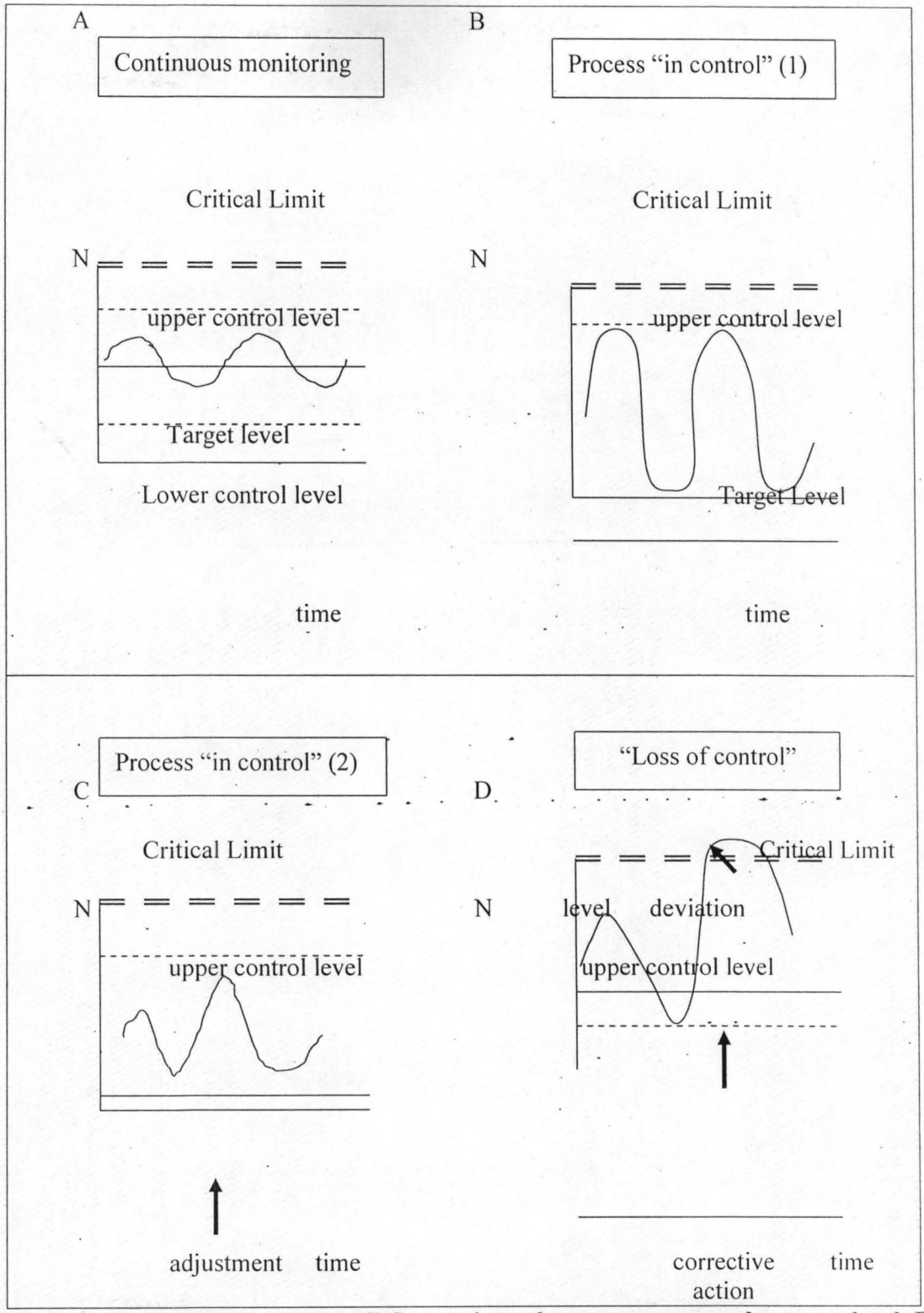


Figure 2.6 – Monitoring: A: small fluctuations always occur around a target level, B and C: the process is under control but adjusted is needed in situation C as abnormal fluctuation are noted, D: a deviation occurs and corrective action is needed (from Mortarjemi and Van sclthorst, 1999).

Step 10: Establish Corrective actions (Principles 5)

Corrective Action is any action to be taken when the results of monitoring at the CCP indicate a loss of control (CAC, 2001) whenever there is a deviation from established critical limits a corrective action must be instituted to ensure that defective products do not reach the consumer. These actions should include the following (NACMCF, 1997):

- determine and correct the cause of deviation
- determine the disposition of products that were produced during the process deviation
- record the corrective action taken

Options for disposition of products placed on hold include:

- isolating and holding products for safety evaluation
- reprocessing
- rejecting and/or destroying of product
- use as by-product (animal feed).

Corrective action procedures should be developed by the HACCP team in advance and specified in the HACCP plan form (Appendix 2). If necessary a more detailed corrective action report should be elaborated including the following information (National Seafood HACCP Alliance, 1997);

- product identification
- description of the deviation
- results of the product evaluation
- corrective action taken including the final disposition of the affected product
- actions to prevent the deviation from recurring
- name of the individual responsible for taking action.

Step 11: Establish verification procedures (Principles 6)

Verification is the application of methods, procedures, tests and other evaluations, in addition to monitoring to determine compliance with the HACCP plan is to prevent food safety hazards from occurring. Verification activities must provide a level of confidence that the HACCP plan is

working properly and is adequate to control hazards. The NACMCF (1997) document is providing guidance on what elements should be included in the verification activitiess:

- Validation – Initial and subsequent validation of the HACCP Plan
  - CCP – record review
  - calibration of instruments
  - targeted sampling and testing
  - microbiological testing
- Verification of the CCP – monitoring
- Review of monitoring, corrective action records
- Comprehensive HACCP system verification.

Thus, the verification procedures include verification of both the individual CCP and the overall HACCP plan. An essential component of verification is validation.

Validation is obtaining evidence that the elements of the HACCP plan are effective (CAC, 2001). Invalidation of the HACCP plan it needs to be established that the plan I scientifically and technically sound. This means that scientifically validation includes review of each part of the HACCP plan from the hazard analysis through to each CCP. The needed information can be obtained from expert advice, scientific studies and literature, in – plant observations and measurements.

Validation - * are the right things done?

* will the system work when put into practice?

Verification -* are the things done right?

* are they done as they were planned to be done?

Apart from the initial validation, subsequent validation as well as materials, product formulation, processing procedures, consumer and handling practices, new confirmation on hazards and their control, consumer complaints, recurring derivations or any other indication that the system is not working. Figure 2.9 shows where validation fits into the process of HACCP implementation.

A periodic comprehensive verification of the HACCP system should be conducted yearly by an unbiased independent authority. This should include a review of the HACCP plan for completeness, confirmation of the flow diagram, review of all records and validations, sampling and testing to verify CCPs (NACMCF, 1997).

Verification is the responsibility of the producer or food handler. However, where regulatory agencies are conducting audits or sampling end-products the results can be used by industry as part of the verification programme.

Verification producers should be entered on the HACCP plan form (figure 2.7) and results into special verification records.

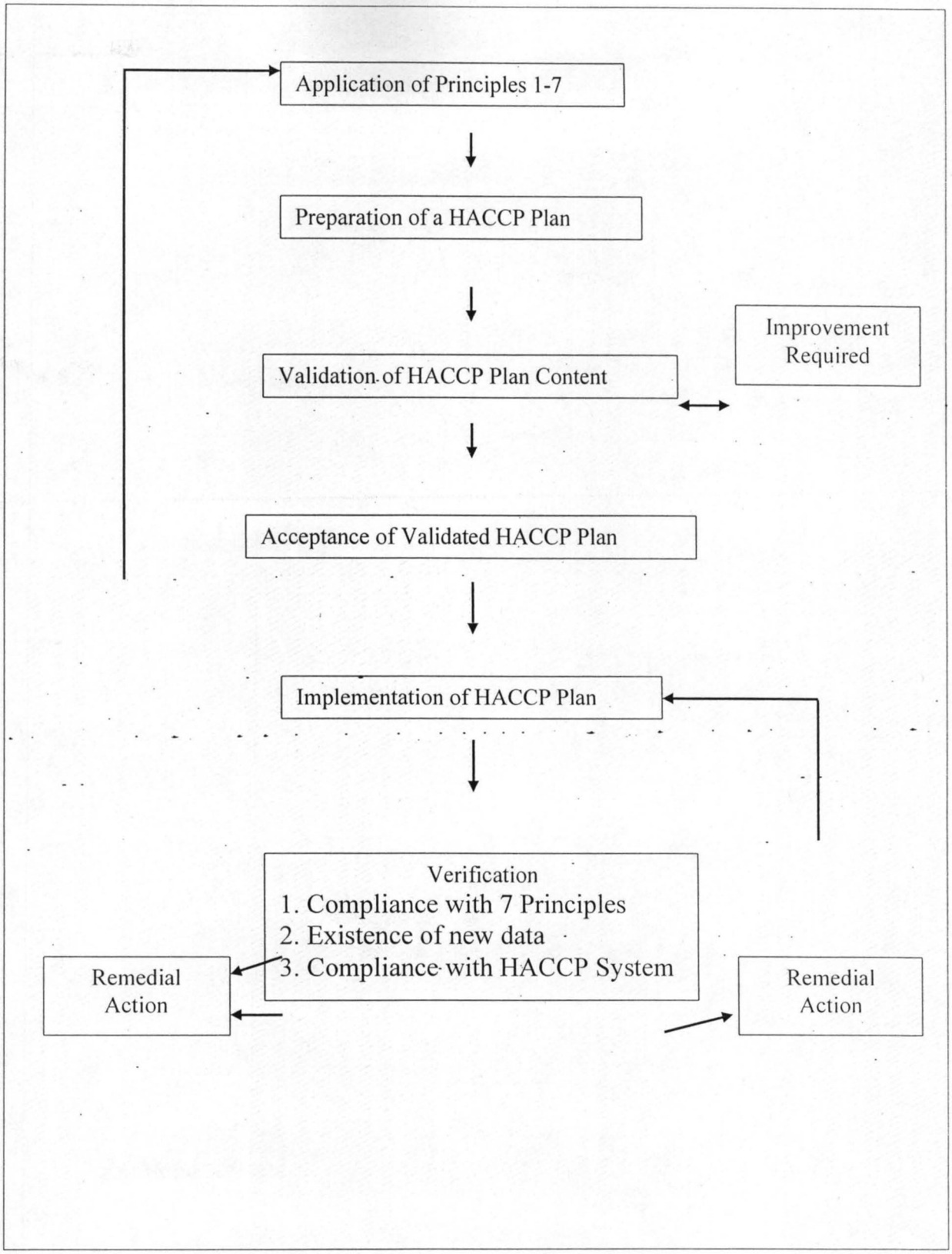


Figure 2.7 – HACCP validation and verification (based on ILSI,1999).

Step 12: Establish record-keeping and documentation procedures (Principle 7)

Record Keeping – ensures that the information resulting from the HACCP study and implementation of the resulting HACCP plan is available for validation, verification, review, auditing and other purposes (ILSI, 1997).

Records and documentation are vial for the verification and auditing to determine if the HACCP system in operation is in compliance with the HACCP plan and operating correctly. Also records of support documents must be kept such as data used to establish critical limits, reports from consultants or experts, a list of the HACCP team and their responsibilities and the preliminary steps taken before development and implementation of the HACCP plan. The CAC (1997) publication mentions the following examples of documentation:

- hazard analysis worksheet
- CCP determination
- Critical limit determination

and as examples of records:

- CCP monitoring activities
- deviations and associated corrective actions
- modifications of the HACCP system

2.12 Consideration in the Application of the HACCP Principles to Seafood Production

The safety of seafood products varies considerably and is influenced by a number of factors such as origin of the fish, microbiological ecology of the product, handling and processing practices and preparation before consumption. Taking most of these aspects into consideration, seafood can conveniently be grouped as shown (modified from HUSS (1994).

- Mollusc Shellfish
- Raw fish to be eaten without any cooking
- Fresh or frozen fish and crustaceans – to be fully cooked before consumption

- Lightly preserved fish products i.e Nacl < 6% in water phase, pH > 5.0. The prescribed storage temperature is < 5° C. This group includes salted, marinated, cold smoked and graved fish.
- Fermented fish, i.e. Nacl < 8% Nacl, pH changing from neutral to acid. Typically, the products are stored at ambient temperature.
- Semi-preserved fish i.e. Nacl > 6% in water phase, or pH < 5, preservatives (sorbate, benzoate, nitrite) may be added. The prescribed storage temperature is < 10°C. This group includes salted and/or marinated fish or caviar, fermented fish after completion of fermentation)
- Mildly heat – processed (pasteurized, cooked, hot smoked) fish products and crustaceans (including pre-cooked, breaded fillets). The prescribed storage temperature is < 5°C
- Heat – processed (sterilized, packed in sealed containers)
- Dried, smoke – dried fish, heavily salted fish. Can be stored at ambient temperatures.

However, the safety of seafood products and processing cannot be studied in isolation. A large number of hazards are related to the pre-harvest situation or the raw material handling and must be under control, when the raw material is received at the processing factory.

2.13 Hazard analysis of raw material

Most fish and shellfish are still extracted from a wild population, but aquaculture is a very fast growing food production system which also supplies a significant proportion of the production. While there are specific safety aspects associated with wild fish caught in the high sea, the intensive husbandry in aquaculture pose new and increased risks. It is imperative that the HACCP principles are extended beyond the factory-gate and applied throughout the total food production chain from harvest to the consumers' plate.

In a general hazard analysis of the pre-harvest conditions for fish and shellfish and the procedures for handling the raw material before being received at the processing plant a number of significant hazards can be identified:

Virus

The presence of viruses in the harvest area is of particular concern in molluscan shellfish because:

- environments where molluscan shell fish are often subject to contamination from sewage which may contain pathogens (bacteria, viruses).
- molluscan shellfish filter and concentrate pathogens that may be present in the water.
- molluscan shellfish are often consumed raw or only particularly cooked.

Thus, the presence or virus is a significant hazard in molluscan shellfish and fish to be eaten raw. The preventive measures is control and monitoring of harvesting areas for faecal pollution.

Biotoxins

Contamination of fish and shellfish with natural toxins from the harvest area can cause serious consumer illness. The toxins accumulate in fish when they feed on marine algae, where the toxins are produced. They occur in fish from the tropical and subtropical area. (ciquatera) and in small fish poisoning (CFP) is a significant hazard, some guidance can be provided by the historical occurrence of the toxins and knowledge about the safety of the reefs from which the fish has been obtained (Huss *et al* 2000).

The preventive measures for the presence of toxins in shellfish are control and classification of shellfish harvesting areas. As a result, shellfish harvesting is only allowed from "safe" waters. The preventive measures for CFP is to ensure that incoming fish have not been caught in an area for which there is a CFP advisory or for which there is a knowledge that CFP is a problem (Huss *et al* 2000).

Biogenic amines

These amines are produced as a result of time/temperature abuse of certain fish species and they can cause illness in consumers. It is therefore a pos-harvest hazard, but very often a pre-receiving hazard introduced during handling on board the fish vessel or during transportation to the plant after landing.

The preventive measure is rapid chilling of fish immediately after capture. Generally, fish should be packed in ice or chilled sea water in less than 12hours after catch or – in case of large fish such as tuna – chilled to an internal temperature of 10°C or less within 6 hours after capture.

Parasite

It is reasonably likely that parasites will be present in significant numbers of wild caught fish species – and certain aquaculture fish if they are fed on an unheated processing waste or by – catch fish. Thus, parasites should be considered a significant hazard and a preventive measure to eliminate parasites must be identified during processing of any particular fish products.

Chemical

Concern for this hazard primarily focus on fish harvested from fish water, estuaries and near shore coastal waters and on fish from aquaculture. Without proper control it would be reasonably likely to expect that unsafe levels of chemicals could be present in the fish, thus representing a significant hazard. Apart from a few acutely toxic chemicals such as mercury, most chemicals are of medium severity from a health perspective (Huss *et al* 2000)

The preventive measures is the presence of government controlled monitoring programme and ensuring that fish have not been harvested fro water that are closed to commercial fishing. For aquaculture fish the preventive measures are full controls of water of chemical contamination of the environment (soil/water) surrounding the aquaculture site, control of water quality and of the feed supply. Only approved agrochemicals and veterinary drugs should be used and only according to manufacturers instructions. Correct withdrawal times must be observed. Table 2-1 summarizes the hazard analysis of the pre-harvest/pre-receiving situation (Huss *et al* 2000)

One of the great problems in ensuring the safety of seafood products is that processors often have no control and no information about the history of the raw material. This is a serious weakness and every effort to overcome this problem must be carried out. The significant hazards associated with the raw material must be identified and controlled before the raw materials are received at the factory. The receiving step is the first CCP in any seafood processing, and the monitoring procedures will mainly be to check documents.

Table 2.1 Hazard analysis of pre-harvest conditions and raw material handling.(modified after Huss et al; 2000)

Organism/ component of concern	Potential hazard		Analysis of hazard			Control		
	Contamination	Growth	Severity	Likely Occurrence	Significant	Govt monitoring Programme	PP[1]	Incl in HACCP plan
Pathogenic bacteria								
Indigenous	-	+	High	High	+	-	-	+
Non-indigenous	+	+	High	High	+	+	+	+
Viruses	+	-	High	High/Low[2]	+/-	+	+	+
Biotoxins	+	-	High	High/Low[2]	+/-	+	-	+
Biogenic amines	-	+	Low	High/Low[2]	+/-	-	-	+
Parasites	+	-	Low	High	+	-	-	+
Chemicals	+	-	Medium	High/Low[2]	+/-	+	-	+

1 PP = Prerequisite Programme

2 Depending on fish/bivalve shellfish species, geographical position and season, the likely occurrence may be high or low.

Table 2. 2 Hazard analysis or processing of bivalve shell fish (modified after Huss et al; 2000)

Organism/ Component of concern	Potential hazard		Analysis of hazard			Control		
	Contamination	Growth	Severity	Likely Occurrence	Significant	Govt monitoring programme	PP[1]	Incl in HACCP plan
Pathogenic bacteria								
Indigenous	+	+	High	High	+	-	-	+
Non-indigenous	+	+	High	High	+	+	+	+
Viruses	+	-	High	High	+	+	+	+
Biotoxins	+	-	High	High	+	+	-	+
Biogenic amines	-	-	-	-	-	-	-	-
Parasites	-	-						
Chemicals	+	-	Medium	High	+	+	-	+

Table 2. 3 Hazard analysis of raw fish to be consumed raw.(modified after Huss et al;2000)

Organism/ Component of concern	Potential hazard		Analysis of hazard			Control		
	Contamination	Growth	Severity	Likely Occurrence	Significant	Govt monitoring programme	PP[1]	Incl in HACCP plan
Pathogenic bacteria								
Indigenous	-	+	High	Low	-			
Non-indigenous	+	-	High	High	+	+	+	+
Viruses	+	-	High	High	+	+	+	+
Biotoxins	+	-	High	High/Low[2]	+/-	(+)	-	+
Biogenic amines	-	+	Low	Low	+			
Parasites	+	-	Low	High	+	-	-	+
Chemicals	+	-	Medium	High/Low[2]	+/-	+	-	+

Table 2.4 Hazard analysis of fresh/frozen fish and crustaceans to be cooked before consumption(modified after Huss et al;2000)

Organism/ Component of concern	Potential hazard		Analysis of hazard			Control		
	Contamination	Growth	Severity	Likely Occurrence	Significant	Govt monitoring programme	PP[1]	Incl in HACCP plan
Pathogenic Bacteria								
Indigenous	-	+	High	Low	-			
Non-indigenous	+	+	High	Low	-			
Viruses	+	-	High	Low	-			
Biotoxins	+	-	High	High/Low[2]	+/-	+	-	+
Biogenic amines	-	+	Low	High/Low[2]	+/-	-	+	+
Parasites	+	-	Low	Low	-			
Chemicals	+	-	Medium	High/Low[2]	+/-	+	-	+

Table: 2.5 FAO/WHO ACCEPTABLE LIMITS OF CHEMICALS AND MICROBES IN FOOD (FISH)

CHEMICAL	PERMISSIBLE LIMITS	DESIRABLE LIMITS
Copper	5.0 – 7.0ppm	1.00ppm
Lead	0.01ppm	0.001ppm
Manganese	0.15ppm	0.001ppm
Iron	0.05ppm	0.001ppm
Zinc	5ppm	1.00ppm
Sulphate	400ppm	100ppm
Hydrogen Sulphate	0.05ppm	0.0001ppm
Aluminium	0.1ppm	0.0001ppm
Tin	0.002ppb	0.0002ppb
Mercury	below 1ppb	0.001ppb
Chromium	0.05ppm	0.0001ppm
Cadmium	0.005ppm	0.0001ppm
MICROBES		
Entrobacterecae sp Such as *shigella sp, Salmonella sp, and coli*	1/100mls	0/100m
Other microbes such as enteroccocci	40/100mls	0/100ml

Fresh/Frozen fish and crustaceans – to be fully cooked before consumption

The hazard analysis of these products is fairly straightforward and complicated. The animals are inmost cases caught in the sea or freshwater, handled and processed without any use of additives or chemical preservatives and finally distributed with chilling or freezing as the only means of preservation.

The epidemiological evidence has shown that the presence of histamine or biotoxins accounts for nearly 80% of all diseases outbreaks caused by "fish". Low levels of pathogenic bacteria and viruses may be present on raw fish as part of the natural flora and/or as a result of contamination during handling and processing. As the product will be cooked before consumption, it is very unlikely that this low level of pathogens will cause any disease. Even if any growth has taken place in the raw fish to be cooked, it is unlikely to produce any disease. Pathogenic bacteria and viruses are therefore not significant hazards, which need to be controlled (Huss *et al* 2000).

In contrast the biotoxins {ciguatoxin and tetrodotoxin} are heat stable and cooking the fish before consumption is not likely to eliminate this hazard. In areas where this hazard is likely to occur, it must be noted as a significant hazard. Similarly the biogenic amines (histamine) are resistant to heat, and if present in the raw fish it is likely to cause disease. Production of histamine in raw fish is therefore a significant hazard that must be controlled.

Parasites are common in fish, but normal household working will kill the parasites, and their possible presence is therefore not a significant hazard.

Chemical contamination of fish is unlikely and not a significant hazard except for aquaculture fish and fish from coastal areas subject to industrial pollution (Huss *et al* 2000).

2.14 DEFINITION OF TERMS

CCP Decision Tree:

A sequence of questions to assist in determining whether a control point is a CCP.

Control:

(a) To manage the conditions of an operation to maintain compliance with established criteria.

(b) The state correct procedures are being followed and criteria are being met.

Control Measure:

Any action or activity that can be used to prevent, eliminate or reduce a significant hazard.

Control Point:

Any step at which biological, chemical, or physical factors can be controlled.

Corrective Action:

Procedure followed when a deviation occurs.

Criterion:

A requirement on which a judgment or decision can be based.

Critical Control Point:

A step at which control can be applied and is essential to prevent or eliminate a food safety hazards or reduce it to an acceptable level.

Critical Limit:

A maximum and/or minimum value to which a biological, chemical or physical parameter must be controlled at a CCP to prevent, eliminate or reduce to an acceptable level the occurrence of a food safety hazards.

Deviation:

Failure to meet a critical limit.

HACCP:

A systematic approach to the identification, evaluation, and control of food safety hazards.

HACCP Plan:

The written document which is based upon the principles of HACCP and which delineates the procedures to be followed.

HACCP System:

The result of the implementation of the HACCP plan.

HACCP Team:

The group of people who are responsible for developing, implementing and maintaining the HACCP system.

Hazard:

A biological, chemical, or physical agent that is reasonably likely to cause illness or injury in the absence of its control.

Hazard Analysis:

The process of collecting and evaluating information on hazards associated with the food under consideration to decide which are significant and must be addressed in the HACCP plan.

Monitor:

To conduct a planned sequence of observations or measurement to assess whether a CCP is under control and to produce an accurate record for feature use in verification.

Pre-requisite Programs:

Procedures, including good manufacturing practices, that address operational conditions providing the foundation for the HACCP system.

Severity:

The seriousness the effect(s) of a hazard.

Step: A point, procedure, operation or stage in the food system from primary production to final consumption.

Validation: That element of verification focused on collecting and evaluating scientific and technical information to determine if the HACCP plan, when properly implemented, will effectively control the hazard

Verifications: Those activities, other than monitoring, that determine the validity of the HACCP plan and that the system is operating according to the plan.

2.15 Cost Associated with Implementing HACCP

Initial/Start up costs

- Formal meetings/management costs.
- Preparation of background information (e.g. flow charts).
- Staff training.
- External consultant fees.
- Overtime/pay costs
- Possible equipment costs (e.g. to layout or fabric of the building), in addition to that needed for monitoring and possible design and construction costs.
- Increased cost of documentation.
- Miscellaneous, e.g. travel costs for training.

Implementation costs

- Time spent on monitoring
- Cost of monitoring, e.g. chemical costs – such as ATP bioluminescence monitoring of cleaning.
- Arguably these costs, which may be incurred are not truly HACCP costs but relate to having adequate PRPs. However they may be incurred at the time of HACCP implementation.
- Time/money spent on better cleaning
- Costs or corrective actions, if this requires disposal of product.
- Ongoing staff training.
- Increased maintenance costs, e.g. refrigeration equipment for better temperature control.
- Time spent on recordkeeping.

Additional time spent on HACCP may not always translate into real or actual costs, e.g. people do more work or substitute HACCP for other works. Overall costs of initiating and implementing

HACCP are affordable even by small business. This is especially true when considered in relation to failure costs, e.g. food poisoning fines, compensation loss of reputation etc.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of Fish Samples

Fresh Tilapia fish samples (64 pieces) were randomly selected and purchased from fishermen and fish sellers at the four designated sites in Minna, Tagwai Dam, Zumba fish market, mobile fish market and chanchaga fish market biweekly between the months of May to July 2009. The fishes were transported with a foiled paper covered with ice-block in stainless steel cooler for analysis.

3.2 Methods

The codex guidelines for the application of the HACCP Principles which follows a series of steps in logical sequence was employed for the method.

3.2.1 Product Description

The fish used in this research work is Tilapia species also known in vernacular as garagaza or kukula. The samples were collected fresh from the fish markets and natural water. Proximate analysis was done to determine the major components of the samples and these components include moisture, lipids (fats) ash (minerals), protein, carbohydrate and fibre.

3.3 Proximate Analysis

Proximate analysis of food is the determination of the major components of food, which include: moisture, lipids (fets) ash (mineral), protein, carbohydrate and fibre.

3.3.1 Determination of Moisture

Indirect distillation method was employed here using drying ovens. The samples were dried in the moisture oven at 70-80°C for two hours and at 100-135°C until weight is constant. The moisture content was then calculated using the equation below

$$\% \text{ Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where: W_1 = initial weight of empty crucible,

W_2 = weight of crucible plus fish sample before drying

W_3 = final weight of crucible plus food after drying

3.3.2 Determination of Ash (Minerals)

Ash constitutes the residue remaining after all the moisture has been removed as well as the organic materials (fats, proteins, carbohydrates, vitamins, organic acids, etc) have been burnt away by igniting at a temperature of around 500°C.

The sample was placed into a pre-heated muffle furnace at 550oC until a lights grey ash results. The percentage ash was then calculated using the formula below:

$$\% \text{ Ash (dry basis)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

3.3.3 Determination of Crude fibre

Crude fibre is made largely of cellulose together with a little lignin. Crude fibre includes theoretically, materials that are indigestible in human and crimal organism.

Procedure

About 5g of sample was placed into 500ml conical flask and 200 ml of boiling 1.25% $H_2 S_{04}$ and then brought to boiling within one minute and allow to boil gently for 30 minutes. It was then filtered through poplin cloth using funnel, and then rinsed well with hot distilled water, after which the material was scrapped back into the flask with a spatula 200 ml of boiling 1.25 % NaoH and few drops of anti foaming agent were then added and brought to boiling within one minutes and allow to boil gently to 30 minutes. It was then filtered through poplin cloth and wash with hot distilled water. It was then rinsed four times with hot distilled water and once with 10% Hcl four times again with hot distilled water, twice with methylated spirit and three times with petroleum ether. The residue was then scrapped into a crucible and dried in oven at 105°C and then cooled in a desiccators and weighed. The crucible was then transferred into a muffle furnace about 300°C for about 30 minutes, and then removed into desicator and allow to cool to room temperature and weighed again. The percentage crude fibre was then calculated using the formula below

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

W_1 = Weight of sample used

W_2 = Weight of crucible plus sample

W_3 = Weight of crucible plus ash

3.3.4 **Determination of Lipids (FAT)**

In general lipids are characterized by their sparing solubility in water and their considerable solubility in organic – solvents. Determination of fat content of a food does not actually reflect the estimation of the true fact content, but of the lipid fraction of the food, that is, those food constituents soluble in non – polar organic solvents such as benzene and petroleum ether.

The soxhlet extraction method was used to determine the lipid content. While the percentage lipid (fat) was calculated thus:

$$\% \text{ Lipid (fat)} = \frac{W_3 - W_4}{W_2} \times 100$$

Where:

W_1 = Weight of filter paper

W_2 = Weight of sample

W_3 = Weight of filter paper plus sample before extraction

W_4 = Weight of fibre paper plus sample after extraction

3.3.5 **Determination of Protein**

Protein are polymers of amino acids and is the only macronutrients in foods that contains nitrogen. The nitrogen in protein thus becomes the basis of the estimation of protein in foods..

Kjeldah method was used in determining the protein. The underlying principle behind this method is the estimation of the total nitrogen in food and the subsequent conversion of the percentage of that nitrogen in food is present as protein. Then using a conversion factor the actual percentage of nitrogen in the food protein is determined. The conversion was done using the simple formula below

% Protein = % Nitrogen X F

Where"

$$F = \text{conversion factor} = \frac{100}{\%\ \text{nitrogen in food protein}}$$

Each food type has it's percentage nitrogen. The common factor used for most foods and food mixture is 6.25 and this was used.

3.4 Identifying and Listing Relevant Hazards and Control Measures

Three major categories of potential hazards were listed namely: physical, chemical and microbiological hazards

3.4.1 Physical Hazards

Identification of physical hazards was done by physically checking fish samples to see if there is cut, wound or abrasion that could create a focal point for infection, skin diseases and looked for the presence of items such as sandglass, wood, stones, metals such as nuts, bolts etc and insects on the sample surface which can be seen physically with the naked-eye.

3.4.2 Chemical Hazards

The fish samples were passed through the process of wet ashing (wet digestion) method due to it's advantage of using lesser temperature of 150°c - 200°c instead of dry ashing which uses a higher temperature (400°c) which could cause some of the heavy metals to evaporate and thus giving a wrong impression of the mineral composition. The digest was then used for the determination of each metal (element) present in the sample using the flame atomic absorption spectrophotometer (model 210/211 VGP).The bulbs for each metal were then used to identify each elements respectively.

3.4.3 Microbiological Hazards

Media Preparation

The samples were analysed for total viable counts using Nutrient agar (NA), Mackconkey agar and Salmonella shigella agar. Each medium was prepared according to the manufacturer's specification and sterilized by autoclaving at 121°c for 15 minutes.

The bacteria isolates were characterized based on cell morphology and biochemical tests. The organisms were identified by comparing their characteristics with those of known taxa as described by Cowan (1974). Some of the biochemical tests carried out are described below:

3.4.3.1 **Gram Staining:**

The smear of each isolate was prepared and fixed, drops of 0.5% crystal violet solution was added to stain it for 1 minute and later replaced with Grams Iodine and allowed to stay for one minute, this was then washed in tap water and decolourized rapidly with 95% ethyl alcohol for about 30 seconds, this was again washed in tap water. The preparation was counterstained with safranin for 30 seconds, then washed under running tap water and blot to dry. The slide was examined under the microscope using oil immersion objective lens (X100).

3.4.3.2 **Motility Test:**

A clean cover slip was held between two fingers and a drop of molten Vaseline was carefully placed on each edge of the cover slip. While still holding the cover slip a drop of the bacterial suspension was applied at the centre of the cover slip were the Vaseline was applied, then the cover slip was quickly and carefully inverted to the cavity slide so that the drop of the bacterial suspension on the cover slip will suspend in the centre of the depression of the cavity slide. The slide was then examined under the microscope using oil immersion (X100) objective lens.

3.4.3.3 **Carbohydrate Fermentation Test:**

.1% peptone water was added to the sterile based fermentation medium and dispensed into test-tubes which were inverted, Durham tube was introduced. This was then inoculated with the bacteria culture and incubated at 37°c for 24 hours. Observation was made after 24 hours for any colour change and gas production.

3.4.3.4 **Catalase Test:**

A drop of hydrogen peroxide (H_2O_2) was placed on a clean grease-free glass slide and a clean sterile rod was used to transfer the organism to the slide. Observation was then immediately made for gas bubbling or effervescence which indicates a positive reaction.

3.4.3.5 **Oxidase Test:**

A sterile filter paper was placed in a sterile Petri-dish and two drops of oxidase reagent was added on the paper, a piece of sterile glass rod was then used to smear the test organism on the filter paper. It was then observed for the appearance of a blue-purple colour within 10 seconds which will indicate a positive reaction. The absence of blue purple colour within 10 seconds indicates a negative reaction.

3.4.3.6 **Indole Test:**

1% peptone water was prepared and inoculated with the bacterial culture and then incubated for 48 hours at 37°c. 0.5ml of Kovac's reagent was then added and shaken gently. Appearanee of red colour was confirmed in some isolates indicating the presence of indole.

3.4.3.7 **Coagulase Test:**

A drop of physiological saline was placed on each end of a slide and a colony of the test organism was emulsified in each of the drops to make two thick suspensions. A drop of plasma was added to one of the suspensions and mixed gently. The substate was then examined for clumping of the organisms within 10 seconds. Clumping indicates positive coagulase test. No plasma was added to the second suspension to differentiate any granular appearance of organism from true coagulase clumping.

3.4.3.8 **Citrate Utilization Test:**

A citrate agar slants was prepared and inoculated with test isolates and incubated at 37°c for 4 days. A colour change from green to blue and growth of the organisms was observed which indicated a positive result.

3.4.3.9 **Methyl Red (M.R) and Voges Proskauer (V.P) Tests**

2ml of sterile glucose phosphate peptone water was inoculated with the bacteria culture in duplicate (A and B) and then incubated at 37°c for 48 hours. 4 drops of methyl red reagent was added to tube, I mix and observe immediately for colour change, while 1ml of 40% KOH and 3ml of 5% alcoholic alpha-naphthol was added to tube B shaken well and observed for colour change.

3.4.3.10 **Starch Hydrolysis**

Each of the isolates were aseptically inoculated on duplicate starch agar plates. Duplicate set of starch agar plates were left uninnoculated to serve as control. The plates were then incubated at 37°c for 48 hours. After incubation the plates (both test and the uninoculated control) were then flooded with grams iodine and observed for halo zones around the test isolates. Uninoculated plates were blue black throughout while the amylase production for the inoculated plates was indicated by holo or transparent zone of clearing around the streaked isolates.

3.4.3.11 **Phosphatase Test:**

Phenolphthalein – phosphate agar plate (1ml of 1% sterile phenolphthalein phosphate + 100ml of molten nutrient agar) was inoculated with test organisms and incubated overnight at 37°c. The culture plate was then exposed to amino vapour, colonies of phosphates positive organism turned pink due to presence of free phenolphthalein.

3.4.3.12 **Urease Production Test:**

Isolates were inoculated in duplicate tubes, leaving the remaining duplicate uninoculated, and all tubes were incubated at 37°c for 72 hours and were examined over 12 hours after 24 hours, in all after 72 hours a colour change from yellow to pink was observed, indicating urease positive.

3.5 Determining the Critical Control Point

Decision tree method was applied here. Decision trees are structured sets of questions which depending upon the answer to one question, directs you towards another question or an

outcome. Critical Control Point Decision tree designed by ILSI, (1997) was applied. This was illustrated in figure 2.6.

3.6 Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA). This was use to evaluate variation in terms of changes in microbial count and chemical parameters at each location and their interactions. Probability level was maintained at 0.05 (confidence limit) (Gomez and Gomez, 1984) while Duncan's Multiple Range Test (DMRT) was used to test significance within the mean of the treatments. (Ignatus, 1986).

CHAPTER FOUR

4.0 RESULTS

4.1 Identification of Hazards and Critical Control Points

The potential hazards noticed in the study area were flies, human and animal waste, and agricultural chemical run off and domestic waste into the water body, the wooden tables on which the fish were displayed were looking dirty and unhygienic, the plastic basins in which the fish were put in were not clean enough, dirts on the body of the fish, the sack used to cover the fishes is also dirty. The critical control points identified were the river, market and environment.

Table 4.1 shows a list of the hazards identified from the analysis of the fish samples.

4.1.1 Physical Hazards Identified

From the observation of the samples from the different locations it was seen that the samples taken from the fish markets which were at the selling point has the following physical hazards, presence of pieces of wood on the body and cut//abrasion. While the samples from landing sites, that is straight from the rivers, hads no physical hazard seen on them.

4.1.2 Chemical Hazards Identified

The presence of the following heavy metals were noticed on all the samples from all the four locations. Cu^{2+}, Pb^{2+} Fe^{3+} and Mn^{2+}.

4.1.3 Microbiological Hazards Identified

The following microbes were isolated and identified on the samples with each occurrence as shown on table 4.1.

Staphylococcus *aureus*

Staphylococcus *pyrogenes*

Pseudomonas *auaeruginosa*

Basillus *subtilis*

Shigella *sonnei*

Micrococcus *luteus*

Shigella *dysenteriae*

Streptococcus *faecalis*

Salmonella *tyhi*

Escherichia *coli*

Proteus *vulgaris*

Bacillus *luteus*

Table 4.1: **List of hazards identified from the analysis of the fish samples**

Samples	Type of hazard	Identified Hazards
1	Physical	Pieces of wood, cut/abrasion.
Fishes from Chanchaga fish market	Chemical	Heavy metals: Cu^{2+} Pb^{2+} Fe^{3+} and Mn^{2+}
	Microbiological	Bacterial identified include; *Staphylococcus aureus* *Staphylococcus pyogenes* *Pseudomonas aerugimosa* *Bacillus subtilis*
2	Physical	Piece of wood
Fishes from Mobil fish market	Chemical	Heavy metals: Cu^{2+} Pb^{2+} Fe^{3+} and Mn^{2+}
	Microbiological	*Staphylococcus aureus* *Bacillus subtilis* *Shigella sonnei* *Micrococcus luteus* *Shigella dysenteriae* *Streptococcus faecalis* *Pseudomonas aeruginosa* *Salmonella typhi*
3	Physical	
Fishes from Tagwai dam landing site	Chemical	Heavy metals: Cu^{2+} Pb^{2+} Fe^{3+} and Mn^{2+}
	Microbiological	*Escherichia coli* *Proteus vulgaris* *Staphylococcus aureus* *Bacillus luteus* *Pseudomonas aeruginosa*
4	Physical	Abrasion: Cu^{2+}, Pb^{2+}
Fishes from Shiroro landing site	Chemical	Fe^{3+} and Mn^{2+}
	Microbiological	*Shigella dysentariae* *Staphylococcus aureus* *Pseudomonas aeruginosa*

Table 4.2: Occurrence and Frequency of Potential Hazards from the four sample stations

Hazards	Station	Frequency of Occurrence
1) Wood	1	12
	2	10
	3	Nil
	4	Nil
2) Cut/Abrasion	1	6
	2	10
	3	Nil
	4	10
3) Cu^{2+}	1	16
	2	16
	3	16
	4	16
4) Pb^{2+}	1	16
	2	16
	3	16
	4	16
5) Fe^{3+}	1	16
	2	16
	3	16
	4	16
6) Mn^{2+}	1	16
	2	16
	3	16
	4	16

Table 4.3: Microbial Occurrence and load from the four stations.

Microbes	Station	Frequency of Occurrence
1) *Staphylococuss spp*	1	4
	2	4
	3	4
	4	4
2) *Bacillus spp*	1	4
	2	4
	3	4
	4	Nil
3) *Pseudomonas spp*	1	3
	2	4
	3	2
	4	3
4) *Shigella spp*	1	Nil
	2	4
	3	Nil
	4	3
5) *Micrococcus spp*	1	Nil
	2	2
	3	Nil
	4	Nil
6) *Streptococcus spp*	1	Nil
	2	2
	3	Nil
	4	Nil
7) *Salmonella spp*	1	Nil
	2	2
	3	Nil
	4	Nil
8) *Proteus spp*	1	Nil
	2	Nil
	3	2
	4	Nil
9) *Escherichia spp*	1	Nil
	2	Nil
	3	2
	4	Nil

Table 4.4: Shows the effect of location on bacterial load.

4.1.4 Effect of Locations on the Bacteria Load

From the statistical analysis it was seen that the first and third sets of samples taken from the four locations shows a significance level of the effect of location on the bacterial load, while the second and fourth sets of samples taken shows no significant difference. (Table 4.4).

Table 4.4: Effect of location on the bacterial load.

Sampling Times	Treatments T_1	T_2	T_3	T_4	X	SD	LS
S_1	$1.5x10^6a$	$1.7x10^4b$	$1.1x10^4b$	$5.5x10^5b$	$5.2x10^5$	$4.6x10^5$	* *
S_2	$5.6x10^5a$	$4.1x10^4a$	$6.3x10^4a$	$1.9x10^4a$	$1.7x10^5$	$4.5x10^5$	NS
S_3	$1.5x10^6a$	$5.9x10^4b$	$7.1x10^4b$	$5.5x10^5b$	$5.4x10^5$	$4.5x10^5$	* *
S_4	$5.0x10^3b$	$4.2x10^4b$	$3.4x10^4b$	$1.0x10^6a$	$2.7x10^5$	$4.2x10^5$	NS
Overall	$9.0x10^5a$	$4.2x10^4b$	$4.5x10^4b$	$5.3x10^5a$			

KEY: * * - Significantly Different

NS - No Significant Difference

T_1 - Chanchaga Fish Market

T_2 - Mobil Fish Market

T_3 - Tagwai Dam Landing Site

T_4 - Shiroro Dam Landing Site

4.2.0: Isolation and Characterization of Isolates from Fish Samples

Bacteria Isolated and identified from the fish samples are shown on table 4.5.

4.2.1 Characterization of Isolates

The Isolates from the fish samples were identified and characterized using the methods described by Cheebrough (2006), Oyeleke & Monye (2008).

...ON OF BACTERIA ISOLATED FROM FISH SAMPLES

S/N	Nature of Sample (Skin + Muscle)	Species of Organisms	Gram Reaction	Cell Shapes	Presence of Spore	Catalase	Coagulase	Motility	Gelatin Hydrolysis	Starch Hydrolysis	Nitrate Reaction	Indole Production	Methyle Red	Urease	Citrate	Voges Prosk +	Oxidase	Hydrogen Sulphate	CARBOHYDRATE FERMENTATION									
																			D. Glucose	Lactose	Sucrose	D-Mannitel	Maltose	Arabinose	Fructose	Xylose	Dulcitol	Sorbitol
1.	C_1 Fi	*Staphylococcus aureus*	-	Cocci	-	+	+	-	+	-	+	-	-	-	-	+	-	-	+	-	+	+	+	-	+	-	-	-
	C_1 Fi	*Pseudomonas aeruguisa*	-	rods	-	+	-	+	+	-	+	-	-	+	+	-	+	-	+	-	+	+	-	-	+	-	-	-
	C_2	*Staphyiococcus aureus*	+	Cocci	-	+	+	-	+	-	+	-	-	-	-	+	-	-	+	-	+	+	+	-	+	-	-	-
	C_3	*Bacillus Subtilis*	+	rods	-	-	-	+	+	+	+	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	-	-
	C_4	* *Staphylococcus pyogenes*	+	Cocci	-	+	+	-	-	-	-	-	+	+	-	-	-	-	+	-	+	-	+	-	+	-	-	-
2.	M_1 "	* *Staphylococcus aureus*	+	Cocci	-	+	+	-	+	-	+	-	-	-	-	+	-	-	+	-	+	+	+	-	+	-	-	-
	M_2 "	*Bacillus subtilis*	+	rods	-	-	-	+	+	+	+	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	-	-
	M_2 "	* *Shigella sonnei*	-	rods	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
	M_3 "	*Micrococcus luteus*	+	Cocci	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	M_3 "	* *Shigella dysenteriae*	-	rods	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
	M_4	* *Streptococcus faecalis*	+	Cocci	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	+	-	-	+	-	+	-	-	-
	" "	*Pseudomonas aeruginosa*	-	rods	-	+	-	+	+	-	+	-	-	+	+	-	-	-	-	-	+	+	-	-	+	-	-	-
	" "	* *Salmonella typhi*	-	rods	-	+	-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	+	+	-	-	+	-	-	-
3.	T_1	* *Escherichia coli*	-	rods	-	+	-	+	-	-	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-
	" "	*Proteus vulgaris*	-	rods	-	+	-	+	+	-	+	+	+	+	+	-	-	+	+	-	-	-	+	-	+	-	+	+
	T_2	*Staphylococcus aureus*	+	Cocci	-	+	+	-	+	-	+	-	-	-	-	+	-	-	+	-	+	+	+	-	+	-	-	-
	" "	Bacillus leutus	+	rods	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Key: - = Negative
+ = Positive

S/N	Nature of Sample (Skin + Muscle)	Species of Organisms	Gram Reaction	Cell Shapes	Presence of Spore	Catalase	Coagulase	Motility	Gelatin Hydrolysis	Starch Hydrolysis	Nitrate Reaction	Indole Production	Methyl Red	Urease	Citrate	Voges Prosk	Oxidase	Hydrogen Sulphate	CARBOHYDRATE FERMENTATION									
																			D. Glucose	Lactose	Sucrose	D-Mannitel	Maltose	Arabinose	Fructose	Xylose	Dulcitol	Sorbitol
	T_3	*Pseudomonas aeruguisa*	-	rods	-	+	-	+	+	-	+	-	-	+	+	-	+	-	-	-	+	+	-	-	+	-	-	-
	T_4	*Staphylococcus aureus*	+	Cocci	-	+	+	-	+	-	+	-	-	-	-	+	-	-	+	-	+	+	+	-	+	-	-	-
4.	S_1	*No growth*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	S_2	*No growth*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	S_3	*' Shigella dysentariae*	-	rods	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	S_4	*Staphylococcus aureus*	+	Cocci	-	+	+	-	+	-	+	-	-	-	-	+	-	-	+	-	+	+	+	-	+	-	-	-
	" "	*Pseudomonas aeruguisa*	-	rods	-	+	-	+	+	-	+	-	-	+	+	-	+	-	-	-	+	+	-	-	+	-	-	-

Key: - = Negative
+ = Positive

T_{1-4} = Tagwai Dam

S_{1-4} = Zumba Market Shiroro

C_{1-4} = Chanchaga Fish Market

M_{1-4} = Mobil Fish Market

4.2.1 Effect of heavy metals on fish samples

The presence of the heavy metals namely Cu^{2+}, Fe^{3+} Mn^{2+} and Pb^{2+} shows significance difference in location as shown on table 4.6 while table 4.7 shows the correlation matrix of the metals.

Table 4.6 **Effect of heavy metals on fish samples**

Parameters	Treatments				-		
	T_1	T_2	T_3	T_4	X	SD	LS
Cu^{2+}	0.158±0.003a	0.145±0.003c	0.110±0.005b	0.095±0.003a	0.127	0.007	* *
Fe^{3+}	9.075±0.073c	7.075±0.073a	6.705±0.006a	7.075±0.02a	7.483	0.093	* *
Mn^{2+}	0.145±0.03b	0.038±0.003a	0.013±0.003a	0.043±0.006a	0.059	0.007	* *
Pb^{2+}	0.150±0.008a	0.293±0.006b	0.365±0.006c	0.358±0.00a	0.291	0.010	* *

KEY: * * - Significantly Different

NS - No Significant Difference

T_1 - Chanchaga Fish Market

T_2 - Mobil Fish Market

T_3 - Tagwai Dam Landing Site

T_4 - Shiroro Dam Landing Site

Table 4.7 **Correlation Matrix of Metals**.

	Cu	Pb	Fe
Pb	- 0.84*		
Fe	0.71*	- 0.95*	
Mn	0.67*	- 0.94*	0.99*

Significance @ $P < 0.05$

4.3.0 **Proximate and Caloric Value of fish**

The Proximate analysis of the samples are shown on table 4.6

Table 4.8: Proximate Analysis of fish samples

Sample No	**Analysis**	**% Compositions**	
S_1	Moisture	71.5%	
Fish samples from	Ash	0.39%	
Tagwai	Crude fibre	0.20%	100.18%
Dam	Lipid (Fat)	13.16%	
	Protein	14.93%	
S_2	Moisture	73.44%	
Fish samples from	Ash	0.3%	
Chanchaga fish	Crude fibre	0.2%	100.27%
Market	Lipid (Fat)	12.28%	
	Protein	14.05%	
S_3	Moisture	77.23%	
Fish samples from	Ash	0.6%	
Shiroro	Crude fibre	0.2%	100.11%
	Lipid (Fat)	8.25%	
	Protein	13.83%	
S_4	Moisture	76.5%	
Fish samples from	Ash	0.42%	
Mobil fish	Crude fibre	0.2%	100.95%
Market	Lipid (Fat)	8.65%	
	Protein	15.18%	

CHAPTER FIVE

5.0 DISCUSSIONS, CONCLUSION AND RECOMMENDATIONS

5.1 Discussions

The HACCP application on Tilapia species purchased from different sources were examined, the result revealed the physical hazards identified as presence of wood, cut/abrasion and dirt on the body of the samples. The presence of these hazards were beyond acceptable limits and is considered a high risk and may be an access for pathogenic microorganisms. This is in agreement with the findings of Gram .L (2001), who reported that the presence of foreign matter or material which should not be there leads to physical defects that are capable of causing injury and trauma. (Danbaba; *et al* 2007) reported that the presence of these hazards in excess of the acceptable limits is considered as high risk. The Chemical hazards identified include the presence of Cu^{2+}, Pb^{2+} Fe^{3+} and Mn^{2+} (Table 4.4). Concern for this hazards primarily focued on fish harvested from fresh water, estuaries and near shore coastal waters and on fish from aquaculture. The presence of these chemicals beyond the tolerable limits may constitute a high risk. Lead (Pb^{2+}) and copper poisoning may result, this is in agreement with Huss et al., (2004) who reported that without proper control it is likely to assume that unsafe levels of chemicals could be present in the fish, thus representing a significant hazard. This also agrees with Scoging (1998) who stated that concentration of toxic phytoplankton as low as 200 cells/ml may produce toxic shellfish. Apart from a few acutely toxic chemicals such as mercury, most chemicals are of medium severity from a health perspective.

The microorganisms found among the hazards identified in the fish samples are the presence of bacteria such as *Staphylococcus aureus, Shigella sonnei Streptococcus faecalis, Pseudomonas aeruginosa, Bacillus subtilis, Shigella dysenteriae, Micrococcus luteus, Salmonella tyhi, Escherichia coli, Bacillus luteus, Proteus vulgeris* with high microbial load. These bacteria are in excess of acceptable limits, and this therefore constitute a

significant hazard if consumed without proper and adequate processing, this result agrees with Huss et al.; (2004) who states that pathogenic bacteria from the aquatic or general environment may be present in low numbers in all fishes at the time of harvest. Some of the microorganisms isolated from the fish samples are of public health importance. The isolation of these organisms *Staphylococcus spp, Bacillus spp, Salmonella spp, Shigella spp and Echeriachia spp* are significance in food borne diseases and they cause some of the known bacterial food borne illnesses. *Staphylococcus* food intoxication is one of the most common food-borne illnesses giving rise to nausea, vomiting, abdominal cramping, prostration and diarrhea. Petal, *et al*, (1976), Mason (1979), Onuarah, *et al*, (1987) and Efiuvwenwere and Akoma (1995 and 1997) all reported the contamination of millet used for Kununzaki production by *Staphylococcus*. The toxins produced by *Staphylococcus spp* are some what heat resistant, and therefore it is possible to have *Staphylococcus* food poisoning. The most important sources of *Staphylococcus* are the human. These buttress the report of WHO (2005), that about 40% of normal human adults harbors these organisms in the nose and throat, hence the finger tips of human are often contaminated with these bacteria. Consequently, when contaminated foods are held for several hours at temperature well above 6.6°C the *Staphylococcus* will grow and produce toxins.

Bacillus cereus are aerobic, Gram – positive spore – forming bacteria which are widely distributed in the environment. The spores are resistant to drying and are easily spread with dust .*B. cereus* can easily be isolated from many foods but typically occurs only in low numbers especially in raw foods (Granum and Baird – Parker, 2000). The genus *Salmonella* is a member of the *Enterobacteriaseae* family. *Salmonellosis* is a leading cause of bacterial enteric disease in both humans and animals (Brenner *et al*, 2000). *Salmonellosis* manifests itself clinically either as the enteric fever syndrome caused by typhoid or paratyphoid strains or as the nontyphoid dependent gastroenteritis. The latter may progress to a more severe systemic infection. Symptoms of non-typhoid salmonellosis include nausea, abdominal cramps, diarrhea with watery and possible mucoid stools, fever

and vomiting appearing 8-72 hours after exposure to the pathogen (D' Aoust, 2000). Systemic spread may occur leading to cardiac and circulatory problems. The infectious dose of *Salmonellae* is, in general, high – typically around 10^6 cells, however much lower infectious doses (10 – 100 cells) are reported if the organism is protected against stomach acidity e.g fat and if the product is eaten by more susceptible groups such as children.

Salmonellae are typically mesophilic bacteria with a global distribution. However, their main reservoir is the gastrointestinal tract of man and animals, including birds. Also, environments, such as water reservoirs, contaminated with human or animal excreta may harbour salmonella. In particular shell fish growing in contaminated waters may accumulate salmonella and raw Oysters have been the cause of Salmonellosis outbreaks (Ahmed, 1991).

Open marine waters are free from *Salmonella* but estuaries and contaminated coastal waters may harbour the pathogen. Also, poor personal hygiene may transmit the organism. *Salmonella* is rarely detected in fish from temperate waters but may occur in tropical waters and on fish and shell fish from such waters. Up to 10 – 15% of fish samples from India and Mexico were positive of *Salmonella* which has also been detected in several crustacean and molluscan products from India and Malaysia (D' Aoust, 2000). There is evidence that specific serotypes of Salmonella are common in fish farms and become part of the indigenous micro flora (Feldhusen, 2000).

Four species of *Shigella* are known all of which are human pathogenic. The genus shigella is very closely related to another Enterobacteriaceae genus, Escherichia. Shigella dysenteriae causes the most severe condition of bacilliary dysentery whereas Sh. Sonnei causes the mildest of the diseases. The infections dose is low, approximately 10 – 100 cells and from 7 hours to 7 days may lapse before symptoms present themselves. These include abdominal pain, vomiting, fever and diarrhea which may contain bloody stools. The disease is an infectious disease. Sh – dysenteriae occurs on the India subcontinent, in Africa, and Asia whereas the mildest of the species, Sh – Sonnei is the most common in the Western

countries (Lampel *et al*, 2000). In children, particularly in developing countries, the disease may be severe and Shigella diarrhea accounts for hundreds of thousands deaths every year. The primary route of infection is the faecal – oral route with person – to – person being the most common route of transmission. Shigellosis outbreaks follow a seasonal pattern with the largest number of outbreaks in the warm (Summer) months.

Unlike *Salmonella*, *Shigella* is not associated with particular food raw materials but its presence is exclusively a question of poor hygienic handling and humans are its natural reservoir. Outbreaks have been caused by a multitude of food products, including Shrimp and Clams (Lampel *et al*; 2000). Shigella are not naturally present in water but may survive for up to 6 months in water (Wachsmuth and Morris, 1989) and may survive for long time in Clams and Oysters (Feldhusen, 2000). Outbreaks have typically involved contamination of raw or previously cooked foods during preparation by an infected, asymptomatic carrier with poor personal hygiene.

The genus Escherichia is a member of the Enterobacteriaceae family and E. Coli is the most common aerobic organism in the intestinal tract of man and warm-blooded animals. Most of the E. Coli strains are harmless commensals that colonise the intestinal tract and probably play important roles in maintaining intestinal physiology. However, some strains of E. Coli are pathogenic and can cause diarrhea disease (Doyle *et al*; 1997).

The main source of E. Coli infections have been (Faecally) contaminated water and contaminated food handlers. Whilst E. Coli is not indigenous to the aquatic environment, it may survive and even multiply in warm tropical waters (Rhoders and Kator, 1988; Jimenez *et al*; 1989) and thus also be isolated from presumed unpolluted waters. All E. Coli strains are mesophilic organisms with optimum growth at 37°C. They do not grow at chill temperatures and are readily destroyed by mild heating.

Although both Salmonella and E. Coli can be isolated from non-contaminated tropical waters, the main source of these organisms and Shigella are human and animal (faecal) contamination. Therefore adherence to Good hygiene practices with emphasis on

clean water and personnel hygiene will control the organisms. As all are sensitive to heating, the GHP – programme must be particularly strict when ready –to- eat foods are processed. Proper treatment (e.g. Chlorination) of water and sanitary disposal of sewage are essential parts in a control programme.

The infectious dose of Shigella and E. Coli is low and thus, it is their mere presence that must be avoided in contrast, most Salmonellae have a higher infectious dose if they are not consumed in very fatty (protective) products. Therefore their growth in the product must be avoided. Growth will be inhibited at chill temperatures and by salting.

Current levels of Salmonella in various foods and its importance in human food-borne infections underline that bacteriological testing and stringent bacteriological standards (e.g. absence) of most foods are insufficient measures in the control of Salmollosis. Even the microbiological quality of harvest water appears not to be a good predictor for Salmonella contamination because Oysters removed from closed and open beds had the same level of contamination (4%) and no correlation was observed between the presence of E. Coli and Salmonella (D'Aoust *et al*; 1980).

Therefore, personal sanitation by food vendors and processors and temperature at which the product is to be kept are considered critical. Holding foods at warm outside temperature for 3 to 6 hours present high safety risk; the risk increase substantially with every hour of holding. Jideani, *et al* (2001) reported that daytime temperature of less than 40°C at midday hours, were conducive for promoting microbial growth.

Bacterial food-borne pathogens are grouped into those that cause food intoxication and those that can result in food-borne bacterial infection.

In case of bacterial food poisoning or intoxication the causative organism multiplies in the food where it produces its toxins. A food poisoning is therefore characterized by rapid on set of the illness (typically symptoms are nausea, vomiting) as the toxins are already formed in the food before consumption. Most often intoxication require that the

toxin producing bacteria have grown to high numbers ($10^5 - 10^8$ cfu/g) in the food before it is eaten.

In contrast, the food merely act as a carrier for the causative organism in food-borne infections. The infectious agent may or may not have multiplied in the food, but the ingested viable bacteria continue to grow within the host's body to produce the typical symptoms (fever, diarrhea). The number of viable bacterial cells necessary to cause disease (the Minimum Infective Dose, MID) varies considerably between bacterial species. Thus the MID is known to be high ($> 10^5 - 10^6$ cells) for pathogenic *Vibrio* spp (Twedt, 1989) and very low *Salmonella typhi* and *Shigella* species (Kothary and Babu, 2001). The MID for pathogens originating in the animal/human reservoir may be high or as low as < 10 organisms for *Shigella* and for E. coli 0157 (Kothary and Babu, 2001). As these bacteria are not normally present in fish and fish products, the main preventive measure is to avoid contamination by applying good hygienic practices (GHP) and good manufacturing practices (GMP). However, some of these bacteria, including *Staphylococcus aureus,* which is a toxin producing bacteria grows in the products and is capable of producing disease.

5.2.0 **Conclusion**

This study showed that hazard analysis critical control points (HACCP) approach in quality control can be employed in the fishing industry. The hazards identified are of great concern, and therefore their identification and documentation will go a long way in contributing to the utilization of fresh fish for consumption. The critical control points identified will be a point for all processors and other interest groups in the fishing industry to take maximum precaution so that safe fish could be made available for consumption, this will in turn increase consumer confidence and higher patronage. The presence of spoilage and contamination by microorganism is an indication that the product (fish) is not produced under good hygienic conditions and practices and thus poses a serious health risk to it's direct consumers. This study also established the need to adopt HACCP in the process of

raw ready –to- eat fish products, and the need for proper policing of this rich and viable sector by regulating agencies.

Low levels of pathogenic bacteria and viruses may be present on raw fish as part of the natural flora and/or as a result of contamination during handling and processing. As the product will be cooked before consumption, it is very unlikely that this low level of pathogens will cause any disease. Even if any growth has taken place in the raw fish to be cooked, it is unlikely to produce any disease. Pathogenic bacteria and viruses are therefore not significant hazards, in the case of fish which wills be cooked before consumption. The HACCP approach therefore has been shown to identify areas of concern where failure has not yet be experienced, making it particularly useful for new operations and processing.

5.3.0 **Recommendations**

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

1) The fish marketers must be properly educated on proper handling of fresh fish to avoid spoilage, and prevent infections/contamination.

2) Government at the Federal level should put in place an effective national food safety program, while the state and local agencies should ensure supervisory and enforcement role.

3) Because the informal food sector is composed o f large, small, and chain units, specific HACCP plans for the fishing industry should be produce and made compulsory for registration of the product by NAFDAC, environmental health agencies and other regulatory body.

4) Awareness must be created down to the fishermen and others along the supply chain through training reinforcement. It may come in the form of video/TV training program: or in the form of work station reminder such as pictorials on hazards associated with each step in the process.

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APPENDIX 1

HAZARD ANALYSIS WORKSHEET (BASED ON NATIONAL SEAFOOD HACCP ALLIANCE, 1997)

) ients ssing ep	(2) Identify potential hazards introduced, controlled at this step	(3) Are any potential food safety hazards significant YES/NO	(4) Justify your decision for column 3	(5) What preventive measure(s) ca be applied to prevent the significant hazards?	Is this step a critical control point? (YES/NO)
	BIOLOGICAL CHEMICAL PHYSICAL				
	BIOLOGICAL CHEMICAL PHYSICAL				
	BIOLOGICAL CHEMICAL PHYSICAL				

Figure 2.7 HACCP PLAN FORM (based on National Seafood HACCP Alliance, 1997)

‹al ‹rol nt ‹P)	(2) Significant Hazards	(3) Critical Limits for each Preventive measure	(4) (5) (6) (7) Monitoring				(8) Corrective Actions(s)	(9) Records	(10) verification
			What	How	Frequency	Who			
							s		