ASPECTS OF MICROBIOLOGY, PHYSICOCHEMICAL AND HAZARD ANALYSIS CRITICAL CONTROL POINTS OF "OGIRI" PRODUCTION FROM DIFFERENT SUBSTRATES

BY

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CHAPTER ONE

1.0 INTRODUCTION

"Ogiri" is an oily paste produced mainly from melon seed and consumed within West African countries (Odufa, 1981a). Apart from melon seed (*Citrullus vulgaris*), which is the regular substrate used for the production of "ogiri", castor oil seed (*Ricinus communis*), climbing melon (*Cucumeropsis manni*) seeds, and fluted pumpkin (*Telfaria occidentalis*) seeds are also used as alternative substrates for the production of "ogiri" (Enujiugha, 2003,and Odibo *et al.,* 1990) The use of alternative substrates for "ogiri" production during the last three decades is as a result of increase in consumption rate as well as the high prices of its substrates (Odibo and Ume, 1989). The alarming increase in the demand and prices of various substrates for "ogiri" production was also reported by David and Aderibigbe, (2010) and Odibo *et al.,* (2012) which resulted in the use of *Cucumeropsis* seeds which are relatively cheaper and unpopular for "ogiri" production used as food condiment. "Ogiri" constitute major soup condiment in Anambra, Ebonyi, Enugu, Abia and Imo States of Nigeria.The production of "ogiri" from these seeds is based on fermentation by species of microorganisms which may be indigenous to the seeds or occur in their production environment (Odufa, 1981,and Dimejesi and Iheukwumere, 2014). The traditional preparation of "ogiri" from melon seeds is by method of uncontrolled fermentation (Achi, 2005) and this involves both the raw seeds after which they are dehulled and boiled again to soften the seeds for fermentation. The softened seeds are wrapped in leaves, kept in sacks and left to ferment at room temperature for three to five days prior to drying and mashing to a smooth paste, the "ogiri". Traditional fermentation of food serves several functions which include enhancement of diet through development of flavour, aroma and texture in food substances, preservation and shelf-life extension through lactic acid, alcohol, acetic acid and alkaline fermentation, enhancement of food quality with protein, amino acids, essential fatty acids and vitamins, improving digestibility and nutrient viability, detoxification of anti nutrient through food fermentation processes and a decrease in cooking time and fuel requirement Stein Kraus, (1995). Dehulling is done manually by local producers and this may introduce a myriad of organisms into the seed prior to fermentation, some of which may be pathogenic and can cause spoilage. This development coupled with unhygienic fermentation and operational environment could result in the production of "ogiri" with variable quality and unacceptable aroma, short shelf life and one that pose health hazard to the consumers. Various bacterial and fungal species have been isolated from "ogiri". Odibo,*et al.* (2012) isolated *Bacillus*,

Serratia, *Pseudomonas*, *Klebsiella*, *Staphylococcus*, *Pediococcus* and *Lactobacillus*. Bacteria genera which include *Bacillus*, *Leuconostic*, *Streptococcus*, *Pediococcus*, *Micrococcus* and *Lactobacillus*species were isolated by Oluwale *and Aderibigbe* (2010) from fermenting *Citrullus vulgaris* seed for "ogiri" production. Dimejesi and Iheukwumere isolated *Erwinia*, *Flavobacterium*, *Micrococcus*, *Staphylococcus*, *Bacillus* and *Shigella* species from fermenting castor oil (*Ricinus communis*) seeds for "ogiri" production. Microorganisms found in "ogiri" are not artificially inoculated but found their way into the "ogiri" through variety of sources which may include air, water used in mixing, leaves used in wrapping, the handlers as well as utensils and equipment used in the processing (Odibo*et al.*, 2012).

The greatest food borne disease hazard now appears to be pathogenic bacteria (Marvin *et al.*, 1984) and constitute major public health problems worldwide (Wester *et al*., 1998). Most food borne disease incidents are caused by mishandling or mistreatment of foods in food service in the home, in public and private institutions or in commercial eating establishments. Food borne disease is an inclusive term for many syndromes typically gastroenteritis, with sudden onset of vomiting or diarrhea or both with accompanying abdominal pains. Some episodes include fever, prostration, shock or neurological symptoms. The principal causes of food borne diseases fall into three categories viz:Metallic or poisonous chemicals,toxins occurring naturally in plants or animals, and microbiological agents, viruses bacteria, protozoa, helminthes, molds or their toxins.

Food borne pathogens remain one of the major public health problems worldwide (Nester *et al.*, 1998). Food borne diseases are endemic in many developing countries and constitute a major cause of mortality in these areas (Adam and Moses, 2005). Various regulatory agencies and organizations such as National Canners Association (NCA), Food and Drug Organization (FDO), Food and Agricultural Organization (FAO), World Health Organization (WHO), International Organization for Standardization (ISO), which is represented by the Standard Organization of Nigeria (SON) and National Agency for Food and Drug Administration and Control (NAFDAC) have been set up to monitor the standards of food. Many producers especially the local producers are virtually ignorant to the compliance of these regulatory bodies as a total quality management tool(Enyinnaya, 2013). The twentieth session of Codex Alimentarus Commission held in Geneva from 28 June to 7 July, 1993 recognized the importance of Hazard Analysis Critical Control Point (HACCP) and adopted guidelines for establishment of HACCP system (ALI NORM 93/13A, Appendix II). These regulatory bodies ensure food safety compliances in food production (Firman and Yapp, 2008, and Henson and Headsmen, 1998).

Hazard Analysis Critical Control Point (HACCP) is a systematic approach to ensure food safety by U.S. National Advisory Committee on Microbiological Criteria for Foods (Enyinnaya, 2013.) HACCP has become synonymous with food safety. It is a worldwide recognized systematic and preventive approach that addresses biological, chemical and physical hazards through anticipation and prevention rather than through end product inspection and testing (Omotongun, 2016). Critical Control Points (CCPs) are located at any step of production process where these hazards can either be prevented, eliminated or reduced to acceptable levels. Examples of control points include thermal processing, chilling, testing ingredients for chemical residues, product formulation control and testing products for metal and biological contaminants.

Public health and food authorities worldwide have promoted the concept of HACCP for providing safe and healthy foods. HACCP is being implemented with some degree of success in large and medium-size food industries. However, much progress has not been made in small businesses where food safety problems are particularly important and better control of the risk is needed (Cadmus *et al*, 2013). The safety and quality of foods domestically produced can be therefore critical since the products also find their way into the open market for sale.

HACCP is achieved through the application of HACCP principles which include:Assembly of HACCP team, Product description, Identification of the intended use of product, Construction of flow diagram, On-site verification of flow diagram, Listing all the potential hazards in each step and conduction of hazards analysis, Determination of Critical Control Points,Establishment of critical limits for each CCP, Establishment of monitoring system for each CCP, Establishment of corrective actions.

HAACP is very advantageous as far as food safety is concerned because of the following:

1. It is science based and systematic, identifies specific hazards and measures for their control to ensure food safety and reduces the reliance on end product inspection and testing.
2. It enhances the responsibility and degree of control at the level of the food industry.
3. It aids inspection by food control regulatory authorities and promotes international trade by increasing buyers' confidence.

Various studies have been documented on "ogiri" viz: Some biochemical and natural changes during fermentation of fluted pumpkin seeds (Achiwehu, 1986a and 1986b; Odibo and Umeh, 1989), castor oil seeds (Anosike and Egwuatu, 1981), microbiology, enzymatic activities and amino acid composition of "ogiri", (Odibo and Umeh, 1989; Odibo *et al*, 2008; Falgan, 2012;

Dimejesi and Iheukwumere, 2014) to mention but a few. However, to the best of my knowledge there has not been any work documented on the Hazard Analysis Critical Control Point on production of "ogiri" from different substrates.

1.1 Statement of the Problems

1. Many potential pathogenic bacterial species have been recovered from "ogiri" samples. Though used as food condiment in cooking, it is expected that the heating will destroy the microorganisms and also the heat-labile toxins elaborated in "ogiri". However, "ogiri" is also used in preparing cold ready-to-eat foods and as such portends great danger to those who relish such ready-to-eat foods.
2. Considering the production of "ogiri" and its unhygienic nature as carried out by the traditional producers or village entrepreneurs, there is every need to employ a management system in which food is addressed through the analysis and control of biological, chemical and physical hazards from raw material production, procurement and handling, to manufacturing/production process, distribution and consumption of the finished product.

1.2 Aim of Study

To establish the Hazard Analysis Critical Control Point of "ogiri" produced from creeping melon, fluted pumpkin, climbing melon and castor oil seeds.

1.3 Specific Objectives

The specific objectives in this study include the following:

1. To determine the prevalent organism(s) in each process step.
2. To determine the potential hazards both biological and non-biological.
3. To analyze the constituents of "ogiri" produced from creeping melon seeds (*Citrullus vulgaris*), castor oil seed (*Ricinus communis*), fluted pumpkin seeds (*Telfairia occidentalis*) and climbing melon (*Cucumeropsis manii*).
4. To device control measures of the hazards.
5. To ensure quality and safety of product through proper handling.

1.4 **Justification for the Study**

The unhygienic process condition of “ogiri” results in the production of “ogiri” of variable quality in aroma, flavour, shelf life and may pose health hazard to the consumers. It is therefore necessary to establish a Hazard Analysis Critical Control Points (HACCP) of “ogiri” production in order to address food safety problems.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Food Condiments

Food condiments are those edible items used in smaller quantities to give flavour or relish to the main food. Food condiments are never the main meal but are like additives added in smaller quantity to food /meal for special purpose or function (Nzelu, 2010). Food that are used for condiments production are numerous and varied. Three decades ago, the major soup condiments for flavour improvement in many parts of Nigeria were "Ogiri" "Okpeghe" and dawa-dawa, Ukpaka or Ugba.Others like pepper, spicy leaves and onions were also used to enhance flavor but these were dominated by the "Ogiri" and dawa-dawa. Fermented food condiments produced mainly from leguminous oil seeds are shown in Fig 2.1

Production of "Ogiri" through the fermentation of these leguminous oil seed gives it the characteristic flavour which enhances the taste of Nigerian traditional soups and sauces used as accomplishments to indigenous starchy root tuber diets.

2.2 Processing and production of Ogiri

The major raw materials for the production of "Ogiri" are castor oil seeds (*Ricinus communis*) and melon seeds(*Citrillus vulgaris*).

During the last three decades, the consumption of Ogiri as well as the prices of its various substrates has increased. In Nigeria, alternative substrate, fluted pumpkin (*Telfairia occidentalis)*seed are used for the production of "Ogiri" (Odibo and Umeh, 1989). As a result of the ever-growing increase in the demand and prices of various substrates for the production of "Ogiri", David and Aderibigbe (2010) and Odibo *et al*(2012) used *Cucumeropsis*(climbing melon) seeds which are relatively cheaper and unpopular seeds for the production of "Ogiri". Also, (Akinyele*et al.,* 2013) reported the microbiological, physiochemical and anti-nutritional properties of melon seeds. Barber and Achiwehu (1988) used castor oil seed for "ogiri" production.

In addition, soyabeans (*Glycine max)* has recently emerged as new raw material for the production of "Ogiri'. Figure 2.1 shows the production process of "Ogiri" using different substrates

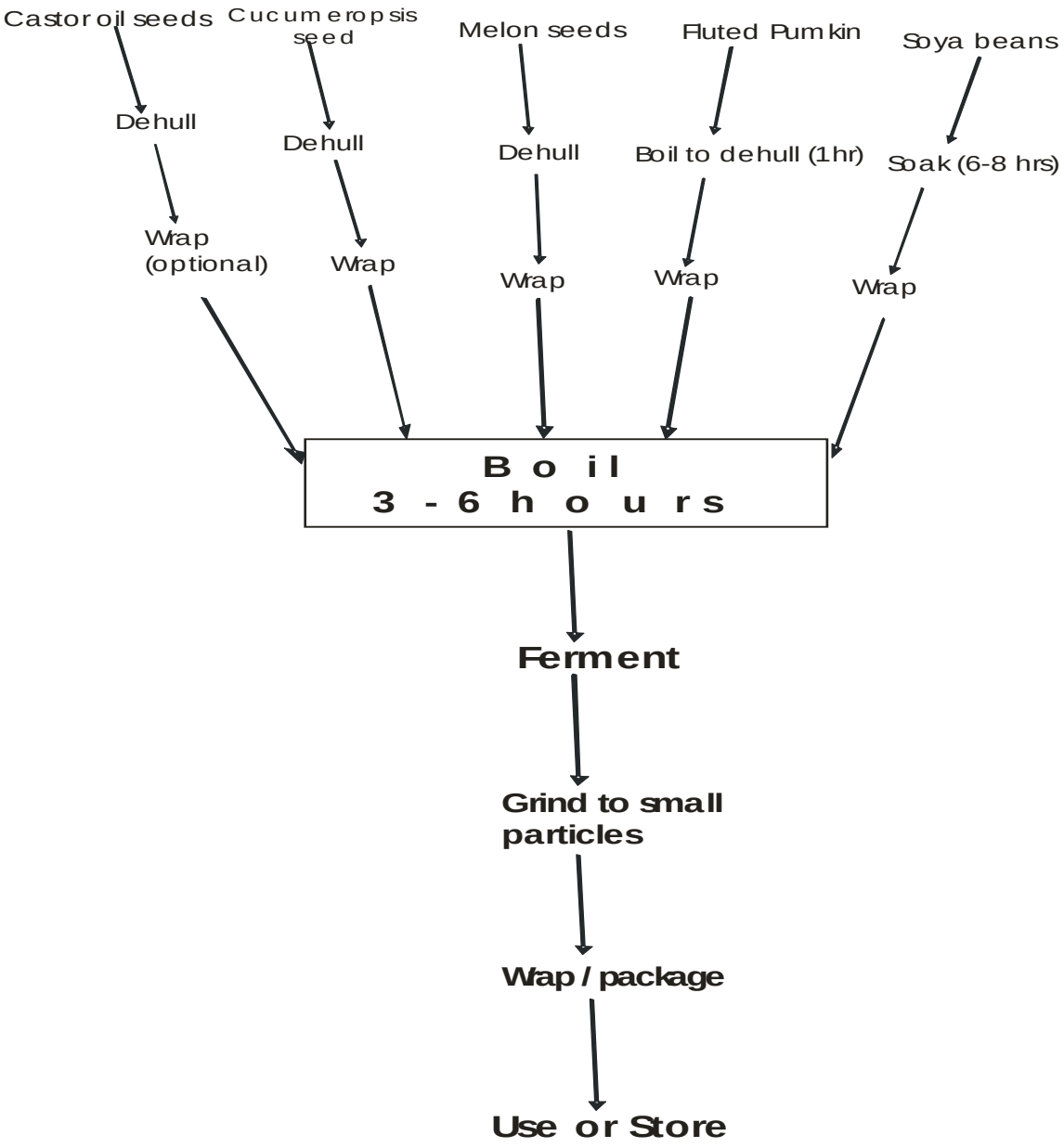


Figure 2.1: The processing steps for the production of "Ogiri" from different substrates source (Nzelu, 2010)

2.3 **Microbiology of Ogiri Production from Different Substrates.**

The organisms found in ‘ogiri’ are not artificially inoculated but found their way into the ‘ogiri’ through variety of sources which may include air, water used in mixing the ‘ogiri’ paste, leaves used in wrapping, the handlers as well as the utensils and equipment used in the processing (Odibo *et al.,* 1988) The micro organisms implicated in the fermentation of fluted pumpkin seeds for ‘ogiri’ production include: *Bacillus* spp, *Staphylococcus aureus, Pseudomonas spp,* and *Lactobacillus* spp (Odibo *et al.,* 1988). Bacterial genera isolated from *Cucumeropsis* seeds include *Bacillus* spp, *Serratia* spp, *Pseudomonas* spp, *Klebsiella* spp, *Staphylococcus* spp, *Pediococcus* spp, and *Lactobacillus* spp (Odibo *et al.,* 2012)

Microorganisms found in castor oil seeds are mostly fermentative. These organisms are usually facultative aerobes which can survive both in the initial aerobic condition and the succeeding anaerobic condition (Odunfa, 1985). Bacterial genera which include: *Bacillus* spp, *Leuconostoc, Streptococcus, Pediococcus,Micrococcus* and *Lactobacillus* have been recovered from fermenting*Citrillus vulgaris* seed for ‘Ogiri’ production (Oluwole *and Aderibigbe.,* 2010; Odumfa, 1981a and b). The isolation of coagulase positive *Staphylococcus aureus,* from fermenting seeds for Ogiri production is of public health concern as the organism is known to cause food poisoning (Frazier and Westhoff 2000) Also the presence of *Klebsiella*, a coliform could constitute a health risk since some species of this genus are associated with disease of man (Collins *et al*, 2004). The high heat treatment given to‘ogiri’ and ‘ogiri okpei’ during cooking destroys this micro organisms and possibly any toxin elaborated in the condiments (Odibo *et al*, 1989 and 1992)

Aspergillus niger and *Saccharomyces spp*were among the fungi isolated from fermenting locust beans.Similarly, *Pencillum spp, Aspergillus niger, Fusarium spp, Saccharomyces spp, Candida spp, and Neurospora spp*, were isolated from raw *Cucumeropsis* seeds. *Penicillum spp, Aspergillus niger,* and *Candida spp* were isolated from raw *Citrullus vulgaris.* (Achiwelu, 1992; Odunfa, 1981a and b).Various microorganisms associated with Nigeria fermented foods are shown in Table 2.1.

Table 2.1 Microorganisms associated with Nigerian fermented foods condiments

SUBSTRATE	MICROORGANISM	PRODUCT
African locust beans *parkiafilicoida*	*Bacillus spp. Pseudomonas spp. Micrococcus spp., Streptococcus*	Iru
Castor seed *Ricinus communis*	*Bacillus spp., E. coil, Staphylococcus spp., Pseudomonas Bacillus subtilis Staphylococcus spp., Micrococcus spp Corynebacterium spp. B. subtilis, B. licheniformis*	Ogiri-igbo
Fluted pumpkin seeds Telferia ocidentalis	*B. megateruim Straphylococcus epidermidis, Micrococcus spp.*	Ogiri-ugu
African oil beans Pentaclethra macrophylla	*Bacillus subtilis, B. licheniformis, B. pumilis, Staphylococcus spp.*	Okphehe
African yam beans *Stenophylis Stenocarpa*	*Saccharomyces cerevisiae, Lactobacillus spp.,Fusarium spp*	Ogiri-egusi

Source: David and Aderingbe (2010)

2.4 Traditional Food Fermentation

Fermentation is one of the oldest and most important food processing and preservation techniques. Food fermentation involves the use of microorganisms and enzymes for the production of food with distinct quality attributes that are quite different from the original agricultural raw material (Awoh, 2008).

Most of the cyanide in cassava tubers is eliminated by fermentation and subsequently during pressing and frying to produce garri. During fermentation, endogenous linamarase present in cassava roots hydrolyze linamarin and lotaustralin releasing hydrogen cynide (HCN). Crushing exposes the cyanogens which are located in the cell vacuole to the enzyme which is located on the outer cell membrane, facilitating their hydrolysis. Apart from garri, thereis a vast array of traditional fermented food produced in Nigeria and other west- African countries as shown in Table 2.2. These include staple foods such as fufu, lafun and ogi, condiments such as 'Iru'(dawadawa, ogiri and ugba(ukpaka), Alcoholic beverage such as "burukutu", pito and fermented milk and cheese are also produced.

The fermentation process of these products constitute a vital body of indigenous knowledge used for food preservation acquired by observations and experience, and passed on from generation to generation.

Apart from increasing the shelf life and reduction in anti-nutritional factors, fermentation markedly improves digestibility, nutritive value and flavour of the raw materials (Katz, 2003).

Microbial growth, either of natural or inoculated populations, causes chemical and/or textural changes to form a product that can be stored for extended periods.Therefore fermentation process also is used to create new, pleasing food flavours and odour (Prescott, 2008).

Detoxification of mycotoxins in foods through Lactic acid bacterial (LAB) fermentation has been demonstrated over the years (Biernasiak *et al*, 2006) and has been successfully used to detoxify cassava toxins (cyanogens) following the fermentation of cassava food.

Fermented maize (Ogi), a staple cereal and a popular weaning food in most rural communities in Nigeria, have been reported to have 50% reduction in aflatoxin B1 after 72 hours of fermentation (Oluwafemi and Ikeokwu, 2015)

2.5 Food Borne Illness

The greatest food-borne disease hazard now appears to be pathogenic bacteria. Although the available statistics are deficient in showing the real incidence of food-borne

illness, they are useful in determining trends and do reflect certain points of interest regarding etiologic agents and their epidemiology (Marvin,, 1984) According to Marvin *et al*(1984), the statistics and other accumulating evidence indicate that most food-borne disease incidents are caused by mishandling or mistreatment of foods in food service in the home, in public, and in private institution or in commercial eating establishments. These data indicates that many people are either careless or uninformed about microbiological hazards of food preparation and handling.

Food-borne disease is an inclusive term for many syndromes. Acute gastroenteritic with sudden onset of vomiting or diarrhea or both, with accompanying abdominal pain is typical. Some episodes includes fever, prostration, shock or neurological symptoms. The incubation period (the time between eating and the onset of first symptom), the type and duration of symptoms vary depending on the etiological agent, the infected individuals genetic predisposition and physical condition, and mediation taken (Marvin, 1984).

The principal cause of food-borne diseases falls into three categories:

1. Metallic or poisonous chemicals
2. Toxins occurring naturally in plants or animals.
3. Microbiological agents, viruses, bacteria, protozoa, helminthes, molds or their toxins.

Food-borne outbreaks caused by chemicals are relatively uncommon metallic poisonings which can occur when high acid foods, as fruit juices and carbonated beverages are stored in, or allowed to flow through metal- containing (copper or cadmium) or metal-coated (zinc, antimony or lead) vessels or pipelines. Other chemical poisoning occur because workers in advertently mistake poisons such as sodium, fluoride or other pesticides for food stuff or condiments, add excessive amounts of flavour intensifier, monosodium glutamate, curing or colour intensifying agents nitrites or preservatives, benzoic acid, to food or in advertently contaminate food when applying pesticides (Marvin, 1984).

Bacterial contamination of food is the most frequent cause of food-borne disease, Many bacterial pathogens, *Salmonella spp, Shigella spp* and some enteropathogenic strains of *Escherichia coli* that are conveyed by food invade the intestinal mucosa and cause true infections, others release enterotoxins during growth or lysis (*Vibriocholerae* some enteropathogenic *E. coli*) or during sporulation (*Clostridium pertringens*) in the gut. Other bacteria such as *Clostridium botulinum* and *Staphylococcus aureus* produce toxin as they proliferate within a food and when the food is eaten, causes an intoxication. Gram positive bacteria may also adhere to the intestinal mucosa and cause diarrhea, *Stayphylococcus*

aureus, *Clostridium pertrimgens* and *Bacillus cereus* have been shown to produce chronic diarrhea by attachment to and colonization of human intestinal mocusa (Marvin, 1984).

Outbreak of hepatitis-A have been traced to several kinds of foods particularly shell-fish (Marvin, 1984). Many food-borne diseases are of unknown etiology. Microbiological studies and epidemiological investigations have established common sources, reservoirs and vehicles of food-borne pathogens.

The diversity of food products and food processing operations affect the number and types of microorganism that may be introduced into food, where they can survive or grow. The following six categories of food-borne disease are listed in Table 2.4:

1. Upper gastro intestinal tract symptoms (nausea, vomiting) occur first or predominate.
2. Lower gastro intestinal tract symptoms (abdominal cramps, diarrhea) occur first or predominate.
3. Sore throat and respiratory tract symptoms
4. Neutrological symptoms (visual, disturbances, vertigo, tingling, paralysis)
5. Allergic symptoms (facial flushing, itching)
6. General infection symptoms (fever, chills, malaise, prostration, aches swollen lymph nodes).

Table 2.2: Guide for Laboratory Test Indicated by Certain Symptoms and Incubation Periods

Incubation periods	Redominant symptoms	Specimen to analyze	Organism, toxin or toxic substances
Less than 1hour	Nausea, vomiting, unusual taste, burning of mouth	Vomitus, urine, blood, stool	Metallic chemicals
1-2 hour	Nausea, vomiting, cyanosis, headache, dizziness, dyspriea, trembling, weakness, loss of consciousness.	Blood	Nitrites
1-6hr, mean 2-4 hr	Nausea, vomiting, retching, diarrhea, abdominal pain, prostration	Vomitus, stool	*Staphylococcus aureus* and its enterotoxins
8-16hr, (2-4 hr rarely)	Vomiting, diarrhea, abdominal cramps, nausea	Vomitus, stool	*Bacillus cereus*
6-24 hr	Nausea, Vomiting, diarrhea, thirst, dilation of pupils, collapse, coma sore throat and respiratory symptoms	Urine, blood	Amanita mushrooms[c]
12-72 hr	Sore throat, fever, nausea, vomiting, rhinorrhea, sometimes a rash	Throat swab	*Streptococcus*
2-5 days	Inflamed throat and nose, spreading grayish exudates, fever, chills, sore throat, malaise, difficulty in swallowing, edema of cervical lymph node	Throat swab, blood	*Corynebacterium, diphtheria*
Lower gastrointestinal tract symptoms (abdominal cramps diarrhea) occur first or predominate			
8-22 hr, mean 10-12 hr	Abdominal cramps, diarrhea, putrefactive, diarrhea associated with *C. perfringens*	Stool	*Clostridium perfringens, Bacillus cereus, Streptococcus faecalis, S. faecium*
12-72 hr, mean 18-36 hr	Abdominal cramps, diarrhea, Fever, chills, malaise	Stool	*Salmonella* (including *S. arizonae*), *Shigella*, Enteropathogenic *Escherichia coli*, other enterobacteriaceae, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Plesiomonas shigelloides*,*Campylobacter jejuni*, *Vibrio cholerae* (01 and non-01), *V. parahaemolyticus*.
3 – 5 days	diarrhea, Fever, vomiting, abdominal pain, respiratory symptoms	Stool	Enteric viruses
1 – 6 weeks	Mucoid diarrhea (fatty stools), abdominal pain, weight loss	Stool	*Giardia lambila*
1 to several weeks, means 3 – 4 weeks	Abdominal pain, diarrhea, constipation, headache, drowsiness, ulcers, variable –	Stool	*Entamoeba histolytica*

	often asymptomatic.		
3 – 6 months	Nervousness, insomnia, hunger pains, anorexia, weight loss, abdominal pain, sometimes gastroenteritis	Stool	*Taenia saginata, T. solium*
	Neurological symptoms (visual disturbances, vertigo, tingling, paralysis)		
Less than 1 hr.	Tingling, and numbness, giddiness, staggering, drowsiness, tightness of throat, incoherent speech, respiratory paralysis.		Shellfish toxin[3.5]
	Gastroenteritis, nervousness, blurred vision, chest pain, cyanosis, twitching, convulsion.	Blood, urine, fat biopsy	Organic phosphate insecticides [5.6]
	Excessive salivation, perspiration, gastroenteritis irregular pulse, pupils constricted, asthmatic breathing.	Urine	Muscaria-type mushrooms[7]
1 – 6 hr.	Tingling, and numbness, gastroenteritis, dizziness, dry mouth, muscular aches, dilated eyes, blurred vision, paralysis, weakness, nausea, vomiting, tingling, loss of weight, confusion.	Blood, urine, stool, gastric washing	Ciguatera toxin chlorinate hydrocarbons (insecticides)
12 – 72 hr	Vertigo, double or blurred vision, loss of reflex to light, difficult in swallowing, speaking and breathing, dry mouth, weakness, respiratory paralysis.	Blood, stool.	*Clostridium botulinum* and its neurotoxins
More than 72 hr.	Numbness. weakness of legs, plastic paralysis, impairment of vision, blindness, coma,	Urine, blood, stool, hair	Organic mercury[5]
	Gastroenteritis leg pain, ungainly high stepping gait, foot and wrist drop.		Triorthocresyl phosphate[5]
	Allergic symptoms (facial flushing, itching)		
Less than 1 hr	Headache, dizziness, nausea, vomiting, peppery taste, burning of throat, facial swelling and flushing, stomach	Vomitus	Histamine

	pain, itching of skin.		
	Numbness around mouth, tingling sensation, flushing, dizziness, headache, nausea,		Monosodium glutamate (Chinese restaurant syndrome)
	Flushing, sensation of warmth, itching, abdominal pain, blood, puffing of face and knees.		Nicotinic acid[5]
	Generalized infection symptoms (fever, chills, malaise, prostration, aches, swollen lymph nodes		
4 to 28 days, mean 9 days	Gastroenteritis, fever, edema about eyes, perspiration, muscular pain, chills, prostration, labored breathing.	Muscle biopsy	*Trichinella spiralis*
7 – 28 days, mean 14 days	Malaise, headache, fever, cough, nausea, vomiting, constipation, abdominal pain, chills, rose, bloody stools	Stool, Blood	*Salmonella typhi*
10 – 13 days	Fever, headache, myalga, rash	Lymph node biopsy blood	*Toxoplasma gondii*
10 – 50 days, means 25 - 30 days	Fever, Malaise, lassitude, anorexia, nausea, abdominal pain jaundice	Urine, blood	Etiological agent not yet isolated, probably viral
Varying periods (depends on specific illness)	Prostration, Malaise, swollen lymph nodes, and other specific symptoms of disease in question (for more information on rarer disease see reference 2).	Blood, Stool, Urine, sputum, Lymph node, gastric washings (one of more, depending on organism)	*Bacillus anthracis, Brucella melitensis, B. abortus, B. suis, Coxiella burnetii, Francisella tularensis, Listeria monocytogenes, Mycobacterium tuberculosis, Mycobacterium* spp, *Pasteurella multocida, Streptobacillus moniliformis, Campylobacter jejuni.*

Source: Marvin, 1984

2.6 Importance of Food fermentation

The primary benefit of fermentation is the conversion of sugars and other carbohydrates to usable end products. According to Steinkraus (1995), the traditional fermentation of foods serves several functions, which includes: enhancement of diet through development of flavour, aroma, and texture in food substrates, preservation and shelf-life extension through lactic acid, alcohol, acetic acid and alkaline fermentation, enhancement of food quality with protein, essential amino acids, essential fatty acids and vitamins, improving digestibility and nutrient viability, detoxification of anti-nutrient through food fermentation processes, and a decrease in cooking time and fuel requirement.

2.6.1 Nutritional Benefits

Fermentation can produce important nutrients or eliminate anti-nutrients. Food can be preserved by fermentation, since fermentation uses up food energy and creates conditions unsuitable for spoilage microorganisms. For instance, in pickling, the acid produced by the dominant organism inhibits the growth of all other microorganisms (Katz, 2003)

Fermenting makes foods more edible by changing chemical compounds, or predigesting, the foods for use. There are extreme examples of poisonous plants like cassava that are converted to edible products by fermenting. Some coffee beans are hulled by a wet fermenting process, as opposed to a dry process (Battcock and Aza-Ali, 1998).

Reduction in anti-nutritional and toxic components in plant foods by fermentation was observed in a research which showed “Cereals, legumes, and tubers that are used for the production of fermented foods may contain significant amounts of antinutritional or toxic components such as phytates, tannins, cyanogenic glycosides, oxalates, saponins, lectins, and inhibitors of enzymes such as alpha-amylase, trypsin, and chymotrypsin. These substances reduce the nutritional value of foods by interfering with the mineral bioavailability and digestibility of proteins and carbohydrates. In natural or pure mixded-culture fermentations of plant foods by yeasts, molds, and bacteria, antinutritional components (e.g phytate in whole wheat breads) can be reduced by up to 50%’ toxic components, such as lectins in tempe and other fermented foods made from beans, can be reduced up to 95%. (Larsson and Sandberg, 1991).

Fermentation increases nutritional values of foods, and allows us to live healthier lives. Here are a few examples:

1. The sprouting of grains, seeds, and nuts, multiplies the amino acid, vitamin, and mineral content and antioxidant qualities of the starting product.

2. Fermented beans are easier for the bodies to digest, like the proteins found in soy beans that are nearly indigestible until fermented (Katz, 2003).
3. Fermented dairy products, like, cheese, yogurt, and kefir, can be consumed by those not able to digest the raw milk, and aid the digestion and well-being for those with lactose intolerance and autism.
4. Porridge made from grains allowed to ferment increases the nutritional values so much that it reduces the risk of disease in children.
5. Probotic supplements (beneficial bacterial cultures for microbial balance in the body) are capable of fighting cancer and other diseases.
6. Vinegar is used to leach out certain flavours and compounds from plant materials to make healthy and tasty additions to the meals.

2.6.2. Health Benefits

Fermented food, enjoyed across the globe, conveys health benefits through lactic acid fermentation. The fermentation process can transform the flavour of food from the plain and mundane to a mouth-puckering sourness enlivened by colonies of beneficial bacteria and enhanced micronutrients.

Studies have revealed that *Lactobacillus rhamnosus* and *L. reuteri* which are common organisms in Nigerian fermented foods like ogi and kununzaki could colonize the vagina, kill viruses, and reduce the risk of infections including bacterial vaginosis (Reid *et al.*, 2010). The therapeutic effects of Lactic Acid Bacteria (LAB) and ogi, including their immunostimulatory effect, are due primarily to changes in the gastrointestinal microflora to suppress the growth of pathogens. Increase in population of LAB in the intestine or vagina reduces the cause of bacterial vaginosis, which is a major risks factor for the contraction of HIV (Reid, 2002a). It also reduces the occurrence of gonorrhoea, Chlamydia, and other sexually transmitted diseases (Reid *et al*, 2001b) and diarrhea (Adebolu *et al*, 2007).

All lactic acid producing bacteria (*E. glactobacillus acidophilus, L. bulgaricus, L. plantarum, L. caret, L. Pentoaceticus, L. brevis* and *L. themophilus*) produce high acidity during fermentation. The lactic acid they produce is effective in inhibiting the growth of other bacteria that may decompose or spoil the food. Despite their complexity, the whole basis of lactic acid fermentation centres on the ability of lactic acid bacteria to produce acid, which then inhibits the growth of other undesirable organisms. Other compounds are important as they improve particular tastes and aromas to the final products. The *L. mesenteroides* initiates growth in vegetables more rapidly over a range of temperatures and salt concentrations than

any other lactic acid bacteria. It produces carbon dioxide and acids which rapidly lower the pH and inhibit the development of undesirable micro organisms.

Over 200 species of bacteria live in gut of humans. These microbes help break down food in the intestines, aid in the digestion process, help fight off disease, and boost the immune system. If we eat nothing but overly processed and hard to digest foods, then the fermentation process occur within the GIT resulting into gas, bloating, diarrhea, and constipation might possibly lead to other diseases like cancer. However, providing the body with predigested foods such as fermented foods will help the existing microbes within to do the job they need to do.

Fermentation is not only a way to preserve certain foods, in some cases it actually adds to the nutrient value of it. Fermented vegetables contain more vitamin C and fermented milk products have ample amounts of B vitamins. The bioavailability of these vitamins also increases with fermentation. Probiotics, or "good bacteria" are also formed through the process of fermentation. Fermented soy products contain more vitamin B_{12} (Chung *et al*, 2010).

The desirable bacteria cause less deterioration of the food by inhibiting the growth of the spoiling types of bacteria. Some fermenting processes lower the pH of foods preventing harmful microorganisms to live with too acidic an environment. Controlled fermentation processes encourage the growth of good bacteria which starves, or fights off, the bad microbes.

The fermentation process can be stopped by other means of preserving, such as, canning (heating), drying, or freezing. Heat (Pasteurization, 63^0C), and low temperatures (freezing, 0^0C or below) stops the fermenting process by slowing, or killing, the preferred microorganisms, and other bacteria. A few undesirable bacteria are not killed by either means, and continue to grow. When the beneficial bacteria are gone, the unfavorable bacteria take over, growing exponentially! This causes rotting, disease, illness, and inedible foods. When the goods gups are present and happy, the food remains edible.

Phytates (phytic acid) are the storage form of phosphorus [a mineral] bound to inositol (a B vitamin) in foods high in fiber (all plant foods), and particularly the fiber of raw whole grains, legumes, seeds, and nuts. Although these foods have high phosphorus content, the phosphates in phytates are not released by human digestion. Phytates, particularly in such raw foods as bran, are a concern because they can bind a portion of the iron, zinc, and calcium in foods, making the minerals unavailable for absorption. When bread is leavened (fermented) by yeast, enzymes degrade phytic acid, and phytates pose no problem. Enzymes,

called phytases, destroy phytates during fermentation processes such as: the yeast-raising of dough, Even a small amount of phytates in food can reduce iron absorption by half (by 50%), but the effect is less marked if a meal is supplemented with ascorbic acid (Vitamin C) which also helps the absorption of zinc and calcium.

Fermented food, enjoyed across the globe, conveys health benefits through lactic acid fermentation. The fermentation process can transform the flavour of food from the plain and mundane to a mouth-puckering sourness enlivened by colonies of beneficial bacteria and enhanced micronutrients. While fermented food like yogurt, sauerkraut and kefir are well-known many other lesser-known foods also benefit from the lactic acid fermentation process. Indeed, virtually every food with a complex or simple sugar content can be successfully fermented (Katz, 2003).

Born of both necessity and practicality, lactic acid fermentation proved to be not only an efficient method of preserving food for our ancestors, but also a critical one. Indeed, fermented food like sauerkraut, cheese, wine, kvass, soured grain porridge and breads often sustained tribes and villages during harsh winters when fresh foods were not available let alone plentiful.

2.6.3 Detoxification

The removal of anti-nutrient from the Nigerian fermented foods is an important step in ensuring that the fermented food is safe to eat. Many fermentation foods contain naturally occurring toxins and anti-nutritional compounds. These can be removed or detoxified by the action of micro-organisms during fermentation for instance, the fermentation process that produces the Sudanese product, kawal, removes the toxins from the leaves of *Cassia obtusitfolia* and fermentation is an important step in ensuring that the fermentated foods are safe to eat.

2.6.4 Mycotoxin Detoxification

Foods and feeds are often contaminated with a number of toxins either naturally or through infestation by microorganisms such as moulds, bacteria and virus. Certain moulds often produce secondary toxic metabolites called mycotoxins. These include fumonisins, ochratoxins A, zearalenone and aflatoxins (Katz, 2003). Several methods are available for degrading toxins from contaminated food,for example, alkaline ammonia treatment to remove mycotoxins from food. However, these methods are harsh to food as they involve the use of chemicals which are potentially harmful to health or may impair or reduce the nutritional

value of foods. Cooking foods does not remove mycotoxins either as most of them are heat stable. Detoxification of mycotoxins in foods through lactic acid bacteria fermentation has been demonstrated over the years (Bienasiak *et al*, 2006). Using LAB fermentation for detoxification is more advantageous in that it is a milder method, which preserves the nutritive value and flavour of de-contaminated food. In addition to this, LAB fermentation irreversibly degrades mycotoxins without leaving any toxic residues. The detoxifying effect is believed to be through toxin binding effect.

In a review, Bankole and Adebanjo (2003), reported that the level of Aflatoxin Bl, B2 and G1 were significantly higher in corn from the high incidence area for human hepatocellular carcinoma and the average daily intake of aflatoxin B1 from the high risk areas was 184.1 g/k aflatoxin. Udoh *et al.* (2000) reported 33% of maize sample from ecological zones of Nigeria contaminated with aflatoxins.

Fermented maize (Ogi) is a staple cereal in Nigeria and it is a popular weaning food in most rural communities in Nigreia. Oluwafemi and Ikeowa (2005) have reported that in fermenting maize to ogi, aflatoxin B1 was reduced by about 50% after 72hours of fermentation. Maize as well as other Nigerian cereals are also important raw materials for both local and commercial beer brewing. Oluwafemi and Taiwo (2003) have shown that the role of *S. cerevisiae* in reducing the pH from 5.2 to 3.7 during fermentation is important in detoxifying aflatoxin B during beer fermentation.

2.7 History of HACCP

The concept of HACCP was developed in the 1960's by the Pillsbury company, while working with NASA and the US Army laboratories to provide safe food for space expeditions. The limitations of end product testing became evident to those who were trying to provide the safest possible food products. In order to ensure that food used for space missions would be safe, almost all the product manufactured would need to be tested. The practice of proactive system of HACCP evolves from these efforts to understand and control food safety failures.

HACCP has been widely used by industry since the late 1970s, and is now internationally recognized as the best system for ensuring food safety. It is endorsed by the Food and Agricultural Organization (FAO) and World Health Organization (WHO) of the United Nations, and, in the United States by the National Advisory Committee on Microbiological criteria for Food (NACMCF). In 1972, HACCP was published and

documented in the USA. In 1985 the National Academy of Science (NAS) recommended the use of HACCP.

HACCP is now internationally recognized for ensuring food safety. The HACCP system is endorsed and became used by Food and Agricultural Organization (FAO), World Health Organization (WHO) of the United Nations and in the United systems by National Advisory Committee for Microbiological Criteria for Food (NACMCF). The HACCP system has also been implemented under regulation in other countries like Europe, Canada, Australia and New Zealand and is a high priority program under Codex Alimantaries, the world food standard authority.

2.7.1 The Need for HACCP

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:H7 and *Salimonella enteritidis* became more widespread (NACMCF, 1997).

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 Alimentarious Commission's adoption of HACCP as the international standard for food safety \(Codex, 2007).

2.7.2 Advantages

HACCP offers a number of advantages.

1. Focuses on identifying and preventing hazards from contaminating food is based on sound science.
2. 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.

3. Places responsibility for ensuring food safety appropriately on the food manufacturer or distributor.
4. Helps food companies compete more effectively in the world market
5. Reduces barriers to international trade (Enyinnaya, 2013).

HACCP Principles

HACCP is a systematic approach to the identification, evaluation, and control of food safety hazards based on the following seven principles:

Principle 1: Conduct a hazard analysis

Principle 2: Determine the critical control points (CCPs)

Principle 3: Establish critical limits.

Principle 4: Establish monitoring procedures

Principle 5: Establish corrective actions

Principle 6: Establish verification procedures.

Principle 7: Establish record-keeping and documentation procedures

(Enyinnaya, 2013)

2.7.3 Guidelines for Application of HACCP Principles

HACCP is a management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw materials production, procurement and handling, to manufacturing, distribution and consumption of the finished product. For successful implementation of a HACCP plan, management must be strongly committed to the HACCP concept. A firm commitment to HACCP by top management provides company employees with a sense of the importance of producing safe food.

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. Prerequisite programs such as current Good Manufacturing Practices (cGMPs) are an essential foundation for the development and implementation of successful HACCP plans. Food safety systems based on the HACCP principles have been successfully applied in food processing plants, retail food stores, and food service operations. The seven principles of HACCP have been universally accepted by government agencies, trade associations and the food industry around the world.

Prerequisite Programmes

The production of safe food products requires that the HACCP system be built upon a solid foundation of prerequisites programs such as GMP and SOP. Each segment of the food industry must provide the conditions necessary to protect food while it is under their control. This has traditionally been accomplished through the application of cGMPs. These conditions and practices are now considered to be prerequisites to the development and implementation of effective HACCP plans. Prerequisites programs provide the basic environmental and operating conditions that are necessary for the production of safe, wholesome food. The Condex Alimentarius General Principles of Food Hygiene described the basic conditions and practices expected for foods intended for international trade. While prerequisite programs may impact upon the safety of a food, they also are concerned with ensuring that foods are wholesome and suitable for consumption. HACCP plans are narrower in scope, being limited to ensuring food is safe to consume.

The existence and effectiveness of prerequisite programs should be assessed during the design and implementation of each HACCP plan. All prerequisite programs should be documented and regularly audited. Prerequisite programs are established and managed separately from the HACCP plan. Certain aspects, however, of a prerequisite program may be incorporated into a HACCP plan. For example, many establishments have preventive maintenance procedures for processing equipment to avoid unexpected equipment failure and loss of production. During the development of a HACCP plan, the HACCP team may decide that the routine maintenance and calibration of an oven should be included in the plan as an activity of verification. This would further ensure that all the food in the oven is cooked to the minimum internal temperature that is necessary for food safety (Enyinnaya, 2013).

Education and Training

The success of a HACCP system depends on educating and training management and employees on the importance of their role in producing safe foods. This should also include information on the control of food-borne hazards related to all stages of the food chain. It is important to recognize that employees must first understand what HACCP is and then learn the skills necessary to make it function properly. Specific training activities should include working instructions and procedures that outline the tasks of employees monitoring each CCP.

Management must provide adequate time for thorough education and training personnel must be given the materials and equipment necessary to perform these tasks. Effective training is an important prerequisite to successful implementation of a HACCP plan (USFDA, USDA, NACMCF, 1992).

Developing a HACCP Plan

The format of HACCP plans will vary. In many cases the plans will be product and process specific. However, some plans may use a unit operations approach. Generic HACCP plans can serve as useful guides in the development of process and product HACCP plans; however, it is essential that the unique conditions within each facility be considered during the development of all components of the HACCP plan.

In the development of a HACCP plan, five preliminary tasks need ot be accomplished before the application of the HACCP principles to a specific product and process. The five preliminary tasks are given in Figure 2.2

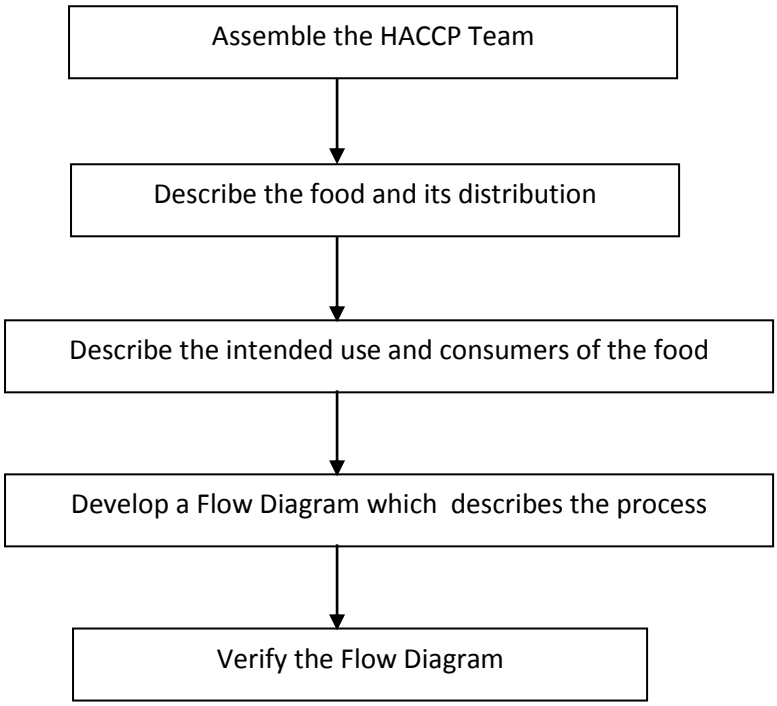


Fig 2.2 Preliminary Task in the development of HACCP

Source: Enyinnaya (2013)

Assemble of the HACCP Team

The first task in developing a HACCP plan is to assemble a HACCP team consisting of individuals who have specific knowledge and expertise appropriate to the product and process. It is the team's responsibility to develop the HACCP plan. The team should be multi disciplinary and include individuals from areas such as engineering, production, sanitation, quality assurance, and food microbiology. The team should also include local personnel who are involved in the operation as they are more familiar with the variability and limitations of the operation. In addition, this fosters a sense of ownership among those who must implement the plan. The HACCP team may need assistance from outside experts who are knowledgeable in the potential biological, chemical and/or physical hazards associated with the product and the process. However, a plan which is developed totally by outside sources may be erroneous, incomplete, and lacking in support at the local level.

Due to the technical nature of the information required for hazard analysis, it is recommended that experts who are knowledgeable in the food process should either participate in or verify the completeness of the hazard analysis and the HACCP plan. Such individuals should have the knowledge and experience to correctly: (a) conduct a hazard analysis; (b) identify potential hazards; (c) identify hazards which must be controlled; (d) recommend controls, critical limits, and procedures for monitoring and verification; (e) recommend appropriate corrective actions when a deviation occurs; (f) recommend research related to the HACCP plan if important information is not known; and (g) validate the HACCP plan (NACMCF, 1992 and Sperber, 1992).

Describe the Food and its Distribution

The HACCP team first describes the food. This consists of a general description of the food, ingredients, and processing methods. The method of distribution should be described along with information on whether the food is to be distributed frozen, refrigerated, or at ambient temperature.

Describe the Intended Use and Consumers of the Food

Describe the normal expected use of the food. The intended consumers may be the general public or a particular segment of the population (e.g infants, immunocompromised individuals, the elderly, etc).

Develop a Flow diagram which Describes the Process

The purpose of a flow diagram is to provide a clear, simple outline of the steps involved in the process. The scope of the flow diagram must cover all the steps in the process which are directly under he control of the establishment. In addition, the flow diagram can include steps in the food chain which are before and after the process that occur in the establishment. The flow diagram need not be as complex as engineering drawings. A block type flow diagram is sufficiently descriptive (see Figure 2.3). Also, a simple schematic of the facility is often useful in understanding and evaluating product and process flow.

Verification of Flow Diagram

The HACCP team should perform an on-site review of the operation to verify the accuracy and completeness of a the flow diagram. Modifications should be made to the flow diagram as necessary and documented.

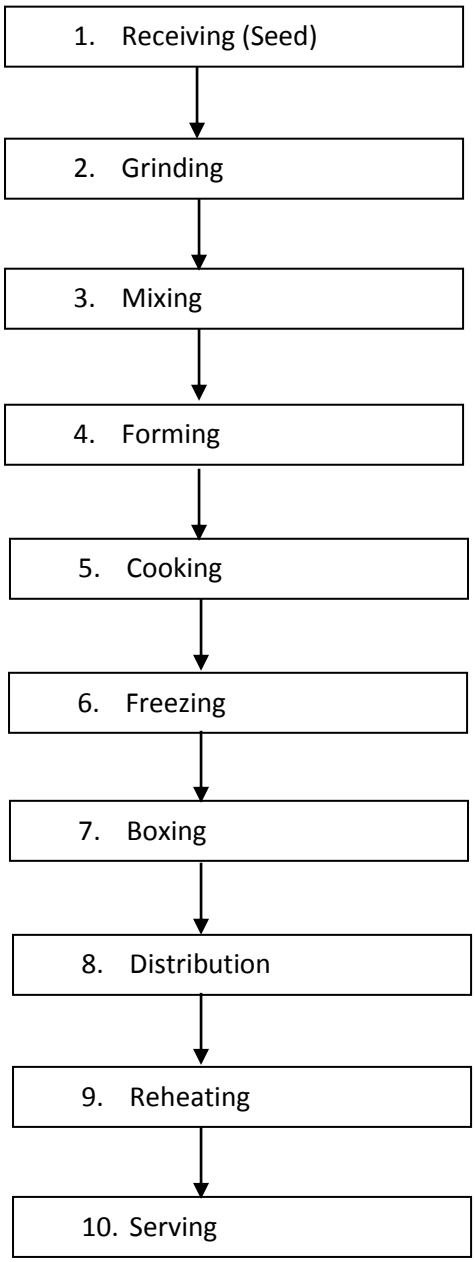


Fig. 2.3 Example of Flow Diagram for the production of frozen cooked beef patties

Source: Okaka and Ene (2005)

Application of HACCP Principle

After these five preliminary tasks have been completed, the seven principles of HACCP are applied.

Conduct a Hazard Analysis (Principle 1)

After addressing the preliminary tasks discussed above, the HACCP team conducts a hazard analysis and identifies appropriate control measures. The purpose of the hazard analysis is to develop a list of hazards which are of such significance that they are reasonably likely to cause injury or illness if not effectively controlled. Hazards that are not reasonably likely to occur would not require further consideration within a HACCP plan. It is important to consider in the hazard analysis the ingredients and raw materials, each step in the process, product storage and distribution, and final preparation and use by the consumer. When conducting a hazard analysis, safety concerns must be differentiated from quality concerns. A hazard is defined as a biological, chemical or physical agent that is reasonably likely to cause illness or injury in the absence of its control. Thus, the word hazard as used in this documents is limited to safety.

A thorough hazard analysis is the key to preparing an effective HACCP plan. If the hazard analysis is not done correctly and the hazards warranting control within the HACCP system are not identified, the plan will not be effective regardless of how well it is followed.

The hazard analysis and identification of associated control measures accomplish three objectives. Those hazards and associated control measures are identified. The analysis may identify needed modifications to a process or product so that product safety is further assured or improved. The analysis provides a basis for determining CCPs in Principle 2.

The process of conducting a hazard analysis involves two stages. The first hazard identification, can be regarded as a brain storming session. During this stage, the HACCP team reviews the ingredients used in the product, the activities conducted at each step in the process and the equipment used, the final product and its method of storage and distribution, and the intended use and consumers of the product. Based on this review, the team develops a list of potential biological, chemical or physical hazards which may be introduced, increased, or controlled at each step in the production process.

After the list of potential hazards is assembled, stage two, the hazard evaluation, is conducted. In stage two of the hazard analysis, the HACCP team decides which potential hazards must be addressed in the HACCP plan. During this stage, each potential hazard is

evaluated based on the severity of the potential hazard and its likely occurrence. Severity is the seriousness of the consequences of exposure to the hazard. Considerations of severity (e.g., impact of sequence, and magnitude and duration of illness or injury) can be helpful in understanding the public health impact of the hazard. Consideration of the likely occurrence is usually based upon a combination of experience, epidemiological data, and information in the technical literature. When conducting the hazard evaluation, it is helpful to consider the likelihood of exposure and severity of the potential consequences if the hazard is not properly controlled. During the evaluation of each potential hazard, the food, its method of preparation, transpiration, storage and persons likely to consume the product should be considered to determine how each of these factors may influence the likely occurrence and severity of the hazard being controlled.

Hazard identified in one operation or facility may not be significant in another operation producing the same or a similar product. For example, due to differences in equipment and/or an effective maintenance program, the probability of metal contamination may be significant in one facility but not in another. A summary of the HACCP team deliberations and the rationale developed during the hazard analysis should be kept for future reference. This information will be useful during reviews and updates of the hazard analysis and the HACCP plan.

Some Questions to be Answered when Conducting a Hazard analysis

Ingredients

1. Does the food contain any sensitive ingredient that may present microbiological hazards such as *Salmonella*, *Staphylococcus aureus*, chemical hazards such as aflatoxin, antibiotic or pesticide residue, physical hazards such as stones, glass, metals?
2. Are potable water, ice and steam used in formulating or in handling the food?
3. What are the sources of e.g geographical region, specific supplier?

Internal factor (Physical characteristics and composition e.g. pH, type of acidulant, fermentation carbohydrates, water activity (Aw), preservatives) of the food during and after processing.

1. What hazard may result if the food composition is not controlled?
2. Does the food permit survival or multiplication of pathogens and/or toxin formulation in the food during processing?

3. Will the food permit survival a multiplication of pathogens and/or toxin formulation during subsequent steps in the food chain?
4. Are there other similar products in the market place? What has been the safety record for these products? What hazards have been associated with the products.

Procedure for processing

1. Does the process include a controllable processing step that destroys pathogens? If so, which pathogens?
2. If the product is subject to recontamination between processing and packaging, which biological, chemical or physical hazards a re likely to occur?

A critical control point is defined as 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. The potential hazards that are reasonably likely to cause illness or injury in the absence of their control must be addressed in determined CCPs.

Complete and accurate identification of CCPs is fundamental to controlling food safety hazards. The information developed during the hazard analysis is essential for the HACCP team in identifying which steps in the process are CCPs. One strategy to facilitate the identification of each CCP is the use of a CCP decision tree. (Enyinnaya, 2013).

Although application of the CCP decisions tree can be useful in determining if a particular step is a CCP for a previously identified hazard, it is merely a tool and not a mandatory element of HACCP. A CCP decision tree is not a substitute or expert knowledge.

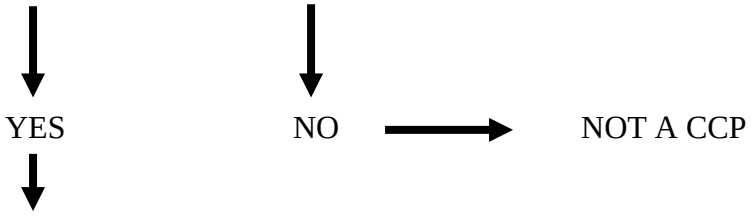
Critical control points are located at any step where hazards can be either prevented, eliminated, or reduced to acceptable levels. Examples of CCPs may include: thermal processing, chilling, testing ingredients for chemical residues, product formulation control, and testing, product for metal contaminants CCPs must be carefully developed and documented. In addition, they must be used only for purposes of product safety. For example, a specific microbiological pathogen, could be a CCP. Likewise, refrigeration of a precooked food to prevent hazardous microorganisms from multiplying, or the adjustment of a food to a pH necessary to prevent toxin formation could also be CCPs. Different facilities preparing similar food items can differ in the hazards identified and the steps which are CCPs. This can be due to differences in each facility’s layout, equipment, selection of ingredients, processes employed, etc.

Establish Critical Limits (Principle 3)

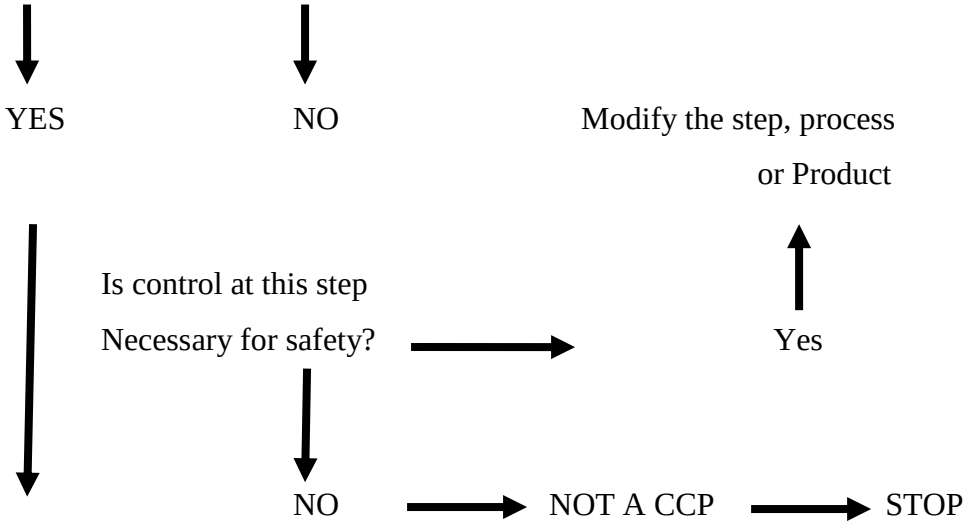
A critical limit is a maximum and/or minimum value to which a biological chemical or physical parameter must be controlled at a CCP to prevent, eliminate or reduce to an acceptable level the occurrence of a food safety hazard. A critical limit is used to distinguish between safe and unsafe operating conditions at a CCP.Critical limits should not be confused with operational limits which are established for reasons other than food safety.

Each CCP will have one or more control measures to assure that the identified hazards are prevented, eliminated or reduced to acceptable levels. Each control measure has one or more associated critical limits. Critical limits may be based upon factors such as: temperature, time physical dimensions, humidity, moisture level, water activity (a_w) pH, titrable acidity, salt concentration, available chlorine, viscosity, preservatives, or sensory information such as aroma and visual appearance. Critical limits must be scientifically based. For each CCP, there is at least one criterion for food safety that is to be met. An example of a criterion is a specific lethality of a cooking process such as a 5D reduction in Salmonella. The critical limits and criteria for food safety may be derived from sources such s regulatory standards and guidelines, literature surveys, experimental results, and experts. The principle can be achieved by the use of decision tree as shown in figure 2.4. Figures 2.5 and 2.6 represent “Fura” and “Ukpaka” production processes showing critical control points (Okaka, 2005).

Q1. Does this step involve a hazard of sufficient risk and severity to warrant its control?



Q2. Does a control measure for the hazard exist at this stage?



Q3. Is control at this stage necessary to prevent, eliminate, or reduce the risk of the hazard to consumers?

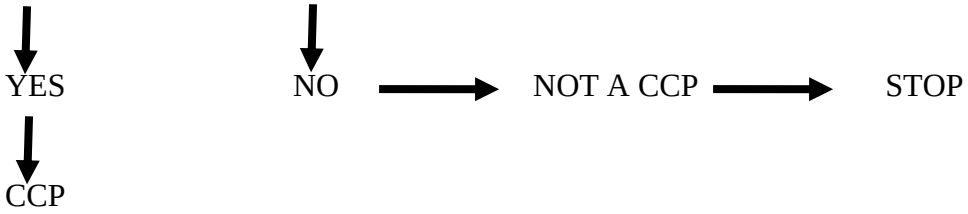


Fig. 2.4 HACCP Decision Tree

Source: Okaka and Ene (2005) and Enyinnaya (2013).

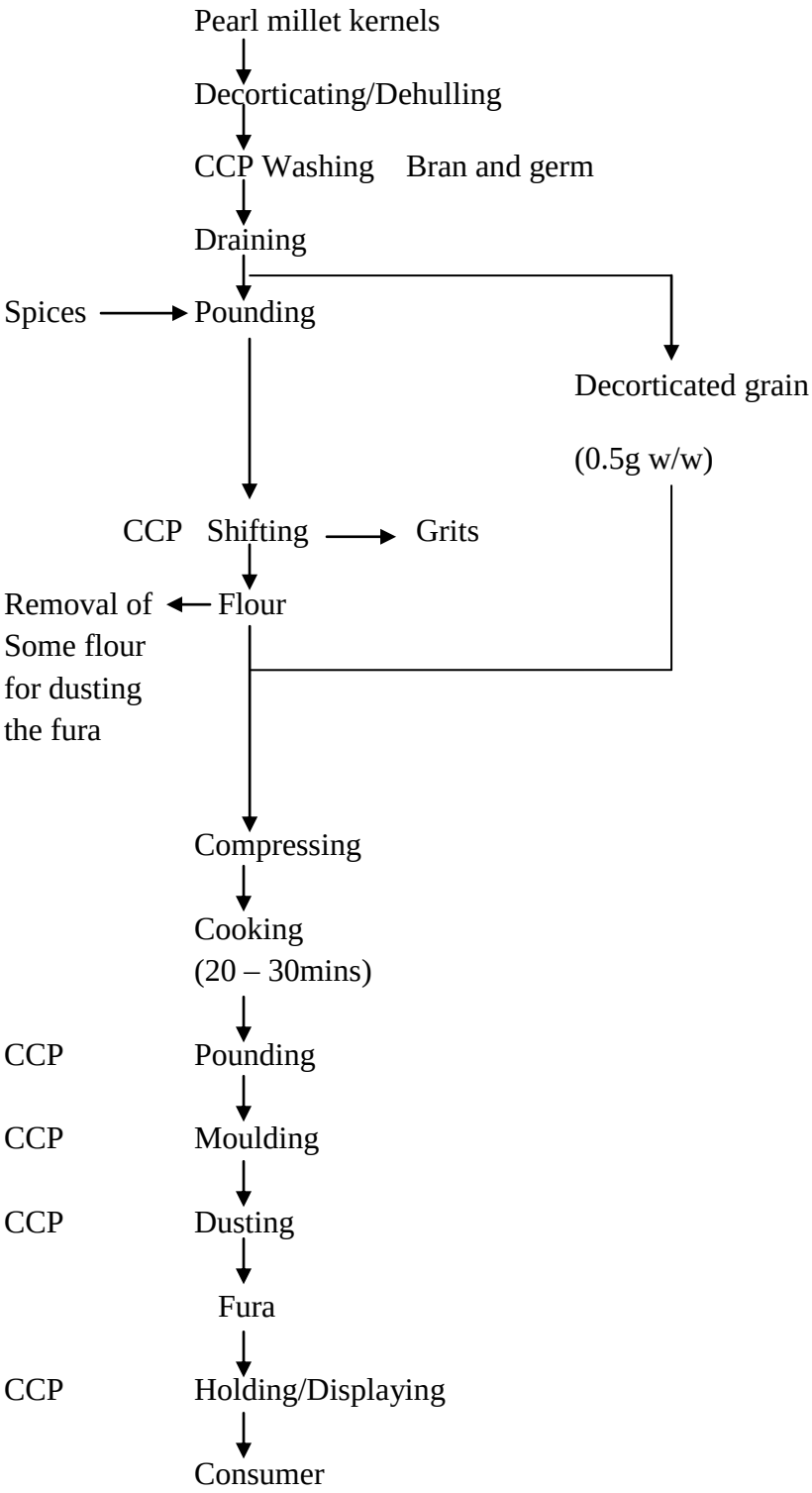


Fig. 2.5 Fura Production Process showing the Critical Control Points (CCP)

Source: Okaka and Ene (2005)

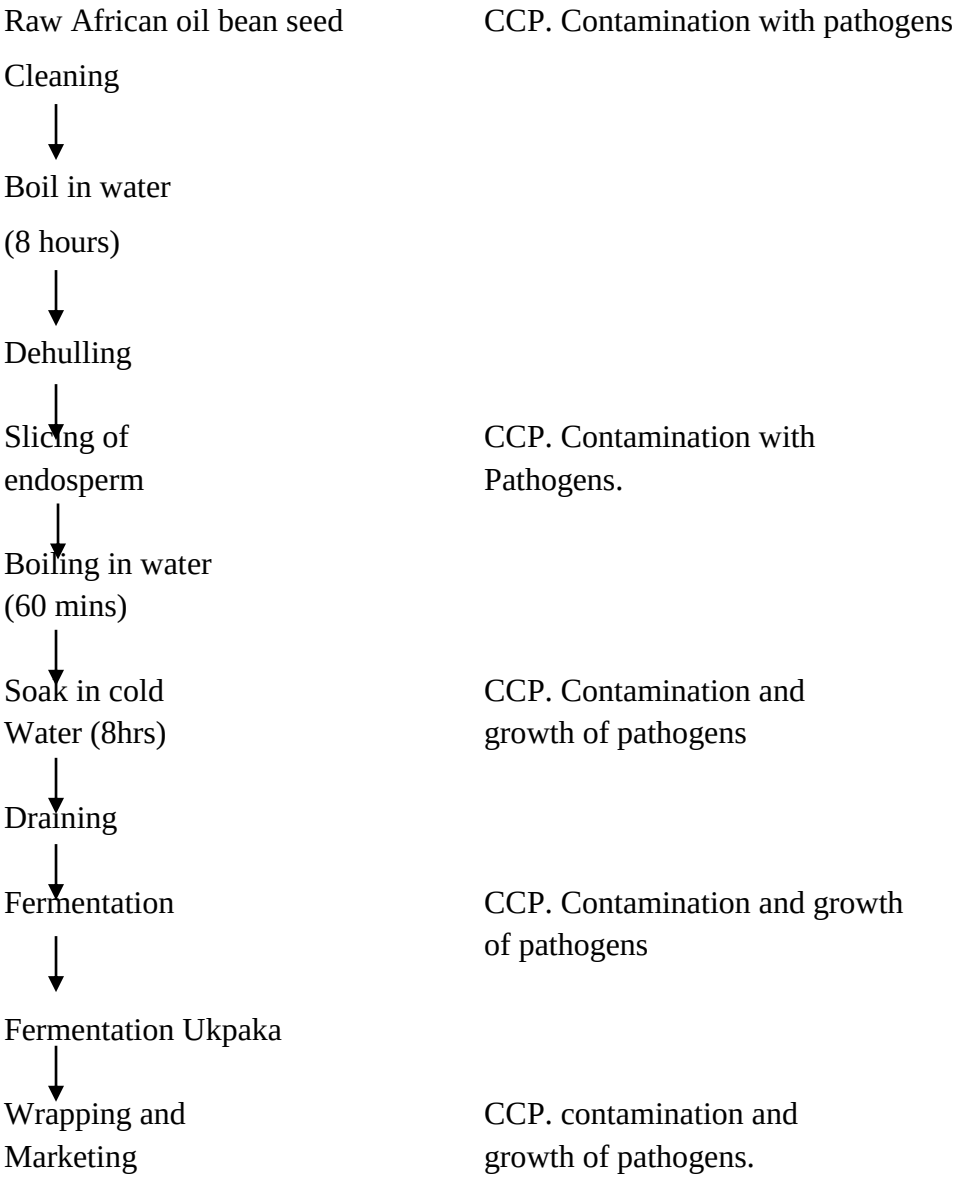


Figure 2.6 Ukpaka production process shown Critical Control Point (CCP)

Source: Okaka and Ene (2005)

Establish Monitoring Procedures (Principle 4)

Monitoring is a planned sequence of observations or measurements to assess whether a CCP is under control and to produce an accurate record for future use of verification. Monitoring serves three main purposes. First, monitoring is essential to food safety management in that it facilitates tracking of the operation. If monitoring indicates that there is a trend towards loss of control, then action can be taken to bring the process back into control before a deviation from a critical limit occurs. Second, monitoring is used to determine whether there is loss of control and a deviation occurs at a CCP, i.e exceeding or not meeting a critical limit. When a deviation occurs, an appropriate corrective action must be taken. Third, it provides written documentation for use in verification.

An unsafe food may result if a process is not properly controlled and a deviation occurs. Because of the potentially serious consequences of a critical limit deviation, monitoring procedures must be effective. Ideally, monitoring should be continuous, which is possible with many types of physical and chemical methods. Assignment of the responsibility for monitoring is an important consideration for each CCP. Specific assignments will depend on the number of CCPs and control measures and the complexity of monitoring. All records and documents associated with CCP monitoring should be dated and signed or initiated by the person doing the monitoring (Enyinnaya, 2013).

Establish Corrective Actions (Principle 5)

The HACCP system for food safety management is designed to identify health hazards and to establish strategies to prevent, eliminate, or reduce their occurrence. However, ideal circumstances do not always prevail and deviations from established processes may occur. An important purposes of corrective actions is to prevent foods which may be hazardous from reaching consumers. Where there is a deviation from established critical limits, corrective actions are necessary. Therefore, corrective actions should include the following elements: (a) determine and correct the cause of non-compliance; (b) determine the disposition of non-compliant product and (c) record the corrective actions that have been taken. Specific corrective actions should be developed in advance for each CCP and included in the HACCP plan. As a minimum, the HACCP plan should specify what is done when a deviation occurs, who is responsible for implementing the corrective actions, and that a record will be developed and maintained of the actions taken. Individuals who have a thorough understanding of the process, product and HACCP plan should be assigned the responsibility for oversight of corrective actions. As appropriate, experts may be consulted to review the

information available and to assist in determining disposition of non-compliant product (Enyinnaya, 2013).

Establish Verification Procedures (Principle 6)

Verification is defined as those activities, other than monitoring, that determine the validity of the HACCP plan and that the system is operating according to the plan. The NAN (1985) pointed out that the major infusion of science in a HACCP system centers on proper identification of the hazards, critical control point, critical limits, and instituting proper verification procedures. These processes should take place during the development and implementation of the HACCP plans and maintenance of the HACCP system.

One aspect of verification is evaluating whether the facility's HACCP system is functioning according to the HACCP plan. An effective HACCP system requires little end-product testing, since sufficient validated safeguards are built in early in the process. Another important aspect of verification is the initial validation of the HACCP plan to determine that the plan is scientifically and technically sound, that all hazards have been identified and that if the HACCP plans is properly implemented these hazards will be effectively controlled. Information needed to validate the HACCP plan often include (1) expert advice and scientific studies and (2) in-plant observations, measurements, and evaluations. In addition, a periodic comprehensive verification of the HACCP system should be conducted by an unbiased, independent authority. Such authorities can be internal or external to the food operation.

Establish Record-Keeping and Documentation Procedures (principle 7)

Generally, the records maintained for the HACCP System should include

1. A summary of the hazard analysis, including the rationale for determining hazards and control measure.
2. The HACCP Plan

Listing of the HACCP team and assigned responsibilities.

Description of the food, its distribution, intended use, and consumer.

Verified flow diagram.

HACCP Plan Summary Table that includes information for:

Steps in the process that are CCPs

The hazard(s) of concern.

Critical limits

Monitoring

Corrective action

Verification procedures and schedule

Record-keeping procedures

A brief summary of position responsible for performing the activity and the procedures and frequency should be provided

The following is an example of a HACCP plan summary table:

CCP	Hazards	Critical limit(s)	Monitoring	Corrective Actions	Verification	Records

2.7.4 Implementation and Maintenance of the HACCP Plan

The successful implementation of a HACCP plan is facilitated by commitment from top management. The next step is to establish a plan that describes the individuals responsible for developing, implementing and maintaining the HACCP system. Initially, the HACCP Coordinator and team are selected and trained as necessary. The team is then responsible for developing the initial plan and coordinating its implementation,. Products teams can be appointed to develop HACCP plans for specific products. An important aspect in developing these teams is to assure that they have appropriate training. The workers who will be repsonsibile for monitoring need to be adequately trained. Upon completion of the HACCP plan, operator procedures, forms and procedures for monitoring and corrective actions are developed. Often it is a good idea to develop a timeline for the activities involved in the initial implementation of the HACCP plan. Implementation of the HACCP system involves the continual application of the monitoring, record-keeping, corrective actions procedures and others activities described in the HACCP plan.

Maintaining an effective HACCP system depends largely on regularly scheduled verification activities. The HACCP plan should be updated and revised as needed. An important aspect of maintaining the HACCP system is to assure that all individuals involved are properly trained so they understand their role and can effectively fulfill their responsibilities.

2.8 Food Safety Hazards

Hazard is a described as any biological or physical property agent that may cause a consumer health risk (Rhodehamel, 1992).

Biological hazard

Biological hazards can be divided into three categories: microorganisms, parasites and prions. It has been reported that microorganisms are the principal sources of food borne disease (Eilers, 1990).

Parasites

Cysticerei, *Tenia* spp, trematodes, nematodes, cestodes, *Trichnilla* spp., *Echinoccocus* spp. and *Aniskis* spp, are commonly isolated parasites from foods. Raw meat and fish are the most risky foods for parasite infections. Conventional cooking destroys them. They may also loss their viability during freezing depending on the time and temperature (Unterimann, 1998).

Prions

Prions are considered as causative agent of "Bovine Spongiform Encephalitis" (BSE). They are resistant to heat treatments and mainly isolated from offal (Untermann, 1998).

Microorganisms

No food product unless it is sterilized is free from microorganisms. Microorganisms of concern in food safety include viruses, some species of bacteria, moulds and protozoa. Of the microorganisms certain species of bacteria and moulds are able to produce toxic metabolites. Their toxigenic effects can range from mild to severe gastrointestinal disorders or chronic syndromes such as carcinogenity, teratogenity, mutagenity and immunosuppression (Adam and Moses, 1999).

Viruses

Hepatitis A, poliovirus, rotavirus, astrovirus and Norwalk viruses are transmitted to man by foods and drinking water. Contamination occurs through faecal oral route (Fries, 1994).

Moulds

Moulds represent health hazards because of their toxic metabolites called mycotoxins. Mycotoxins may be produced in some plants in the field or during drying and storage under improper conditions.

Algae

Some types of algae such as Cyanobacteria (blue green algae) and Pyrophyta (dinoflagellates) produce toxic compounds for human being. Poisoning occur after consumption of some seafood fed with these toxic algae (Falconer, 1993).

Bacteria

Food borne disease caused by performed toxins are referred to food poisoning (intoxications), whereas the disorders caused by bacterial growth or endotoxin production in the body after ingestion were called food infections (Table 2.5)

Table 2.3 Food Borne Diseases

Intoxication/Infection	Bacteria
Infections	*Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia enterocolitica*, *Listeria monocytogenes*, pathogenic *E. coli*, *Aeromonas* spp., *Vibrio cholerae*, *Bacillus cereus* (diarhoagenic type), *Clostridium perfringens*
Intoxications	*Clostridium botulimun*, *B. cereus* (emetic type), *Staphylococcus aureus*

Source: Eley, 1992; Frazier and Westhoff, 1988; Granum *et al*, 1995

Symptoms of the food borne diseases vary with the type of organisms and are more severe in immuno-compromised person, children and elderly, infective doses of bacteria range between 10 to 10^8 cells/g depending on bacterial strain, type of food consumed together with implicated one and health status of consumer (Granum *et al*, 1995).

Bacillus cereus: Some strains of *B. cereus* produce diarrhoagenic and emetic toxins. Emetic toxin is secreted into food and stable to heat treatments above 120^0C (Moss, 1987). Spices, cereals, dried foods, meat and meat products, fried or boiled rice, ice cream, cooked rice dishes, soups, green vegetables, sauces (Kramer and Gilbert, 1989) are the implicated food commodities in *B. cereus* contamination. However, commonly associated foods with emetic and diarrhoagenic syndromes are cooked rice and pertinacious foods respectively (Notermans and Batt, 1998).

Campylobacter spp: *Campylobacter jejuni* and *C. coli* are the major species of *Campylobacters* causing food poisonings and considered as the common causes of gastrointestinal diseases. Most commonly associated foods with *C. jejuni* contaminations are poultry and poultry products (Bryan and Doyle, 1995).

Clostridium spp: *Clostridium botulimum* and *C. perfringens* are the well-known toxin producing species of the genus. The *C. botulinum* represents health hazard rather than its frequency incidence than its deadly antigenic types of botulin toxins (A, B, C1, C2, D, E, F and G) and may be classified based on the type of toxin produced. (Dodd and Austin, 1997). The botulism caused by *C. botulinum* strains is divided into four categories: food borne, infant, wound and those classified as underdetermined. Symptoms of food borne botulism involving nausea, vomiting, weakness, headache, dryness in the throat, double vision and difficulty in swallowing and speaking exists usually 12-72 hours (Hatheway, 1990).. Ingestion of *C. botulinum* spores mainly by honey is the cause of infant botulism. The causes of botulism outbreaks are improperly canned or preserved food (Delmas *et al*, 1994).

Clostridium perfringens is one of the causes of gastroenteritis outbreaks. At least 12 different toxins were produced by *Clostridium perfringens* strains. *C. perfringens* Type A is one of the most common causes of food borne diseases associated with diarrhoeal and cramping symptoms in the USA (McClane, 1992). Beta-toxin produced by *Clostridium perfringens* type C is responsible for a deadly food poisoning known as necrotic enteritis (Granum, 1990). Uncured meat and meat products, sauces, salads, particularly inadequately cooled ones were implicated in *C. perfringens* gastroenteritis (Labbe and Huang, 1995).

Pathogenic *Escherichia coli*: Pathogenic *E. coli* strains are classified into specific groups: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E.*

coli (EIEC), diffuse-adhering *E. coli* (DAEC), enteroaggregative *E. coli* (EaggEC), enerohemorrhagic *E. coli*. Serotype 0157:H7 (Doyle *et al*., 1997). Pathogenic *E. coli* strains were associated with diarrhoeal diseases, wound infections, meningitis, septicaemia, arterioscleroses, haemolytic ureamic syndrome and immunological diseases (Olysvik *et al*, 1991). The main reservoir for *E. coli* 0157:H7 serotypes were reported to be bovine or other foods cross contaminated by beef product or bovine manure (Pierard *et al*, 1997).

Foods involved in pathogenic *E. coil* outbreaks mainly cheese, water, turkey, mayonnaise, crabmeat, scallops, meat, hamburgers, and beef sandwiches (Olsvik *et al*, 1991).

Salmonella spp: *Salmonellae* are considered as one of the most important and common food borne diseases in several counties worldwide. Gastroenteritis, dysentery, enteritis and typhoid are the symptoms of *Salmonella* infections. Meats, poultry and their products are commonly associated foods with *Salmonella* infections (Bryan and Doyle, 1995).

Shigella spp: *Shigella* dysenteriae, *S. flexneri*, *S. boydii* and *S. sonnei* are the pathogenic strains of genus *Shigella* and regarded as the principal causes bacillary dysentery (Vargas *et al*, 1999). Contaminated raw vegetables and fruits, soiled hands, seafood, milk and puddings are the main sources of *Shigella* spp. (Farber, 1989).

Staphylococcus aureus: Medical expenses and loss of productivity due to food poisoning caused by *S. Aureaus* were estimated to cost $1.5billion each year in the United States (Su and Lee Wong, 1997). Enterotoxins are secreted into the foods and resistant to almost all food processing procedures (Moss, 1996). Vomiting, Nausea, abdominal cramps, prostration and some diarrhea usually occur after 2-4 hours following ingestion of contaminated food. Food frequently involvedin *S. aureus* poisoning include meat, poultry, eggs, dairy products, and seafood (Reed, 1993). Man is the main reservoir of post-process contaminations of foods (Reed, 1993).

Listeria monocytogenes: *Listeria monocytoenes* is the causative bacterium of listeriosis and has been linked mainly with flu-like illness, abortion and stillbirth in pregnant women. *L. monocytogenes* also causes septicemia and meningitis in immune-compromised persons. Healthy individuals are not affected in the presence of low numbers of *L. monocytogenes*. Foods commonly implicated with *L. monocytogenes* contaminations are raw milk, fresh vegetables (Schothorst, 1999).

Other Bacteria

Symptoms of *Vibrio cholerae* infections involve loss of water at high levels that may lead to collapse and death. Human can also transmit the bacteria the others as well as foods.

The reservoir of *V. parahaemolyticus* is water, seafood and fish and foods contaminated with water (Schothorst, 1999). There exists some reports indicating intoxications developing with watery diarrhea due to the consumption of foods contaminated with *Yersinia enterocolitica*. Pigs are the main reservoir of *Y. enterocolitica* (Frazier and Westhoff, 1988). Motile groups of *Aeromonas* genus were shown to be responsible for food borne illnesses from mild diarrhea to 'cholera-like' and a 'dysentery-like' nature gastroenteritis (Stelma, 1988). Water was considered as one of the sources of *Aeromonas* spp. (Stern *et al*, 1987).

Control of Microbial Hazards in Foods

The main control measures in order to control microbial hazards in foods are given below:

1. Prevention of contaminations
2. Limitation of microbial growth
3. Elimination/destruction of microorganisms

Control Methods for Microbial Hazards are Tabulated in Table 2.6

Table 2.4 Control of microbial hazards

Heat treatment
Pasteurization
Sterilization
Chemical preservatives
pH
Water activity
Prevention of contaminations
Personnel hygiene
Packaging
Raw material control
Process sanitation
Modified atmosphere
Radiation

Source: Enyinnaya (2013)

Chemical Hazards

Chemical contaminants in food may be naturally occurring or may be added during processing of food. Food may contain several types of chemicals that are hazardous for men. Limits have been established for some types of chemicals whereas others are not allowed to be found in foods (Rhodehamel, 1992). Chemical hazards for foods have been presented in Table 2.7

Table 2.5 Chemical Hazards in various Food.

1. Chemicals naturally occurred in foods
    - Mycotoxins
    - Mushrooms
    - Shellfish toxins
        - PSP (“paralytic shellfish poisoning”) toksinleri
        - DSP (“Diarrhaeic shellfish poisoning”) toksinleri
        - NSP (“Neurotoxic shellfish poisoning”) toskinleri
        - ASP (“Amnesic shellfish poisoning”) toksinleri
    - Scrambotoxin
    - Tetratoksin
    - Ciguatoxin
    - Allergens
2. Chemicals used in the field
    - Pesticides
    - Antibiotics and growth hormones
    - Fertilizers
3. Environmental contaminants
    - Toxic minerals
    - Polychlorinated biphenyls (PCBs)
4. Food additives
5. Chemicals occurred during processing
    - Poycylic Aromatic Hydrocarbons (PAH)
    - N-Nitrosamines
6. Contaminants from food packaging materials
7. Detergent, disinfection residues, chemicals intentionally added
8. From packaging materials – plasticizers, vinyl chloride, printing coatings inks, adhesives, lead, tin

Source: Rhodehamel (1992)

Mycooxins

Mycotoxins are secondary metabolites of moulds. Aflatoxins (B1, B2, G2, G2), fumonisins, moniliformin, ochratoxin *A*, *Patulin*, sterigmatocystine, trichothecenes, zearalenone, alternariols, altertoxins, deoxinivalenol and T-2 toxin are the most commonly isolated mycotoxins. Limit values for some mycotoxins are given in Table 2.8

Table 2.6 Established Limits for some common types of mycotoxins

Mycotoxins	Established limits (ppb)
Aflatoxins in food	0-50
Aflatoxin in milk (M_1)	0-0,5
Ochratoxin A	1-300
Patulin in apple juice	20-50
T-2 toxin	100

Source: Moss (1996)

Studies on mycotoxins have been concentrated on aflatoxins, which are considered as potential carcinogens. Fumonisins have received great attention in recent years; epidemiological studies indicated a correlation between eusophagu cancer and consumption of foods contaminated with fumonisins. Ochratoxin A is commonly isolated from coffee grains and barley. Patulin is a common problem in apple juice all over the world.

Shellfish Toxins

Shellfish toxins cause "Paralytic Shellfish Poisoning" (PSP), "Diarrhetic Shellfish Poisoning" (DSP), "Emnesic Shellfish Poisoning" (ASP) and "Neutrotoxic Shellfish Poisoning" (NSP) through shellfish fed by toxic algae (Falconer, 1993).

Allergens

Some chemicals in foods and additives can cause allergic reactions in susceptible individuals, and are considered as component of HACCP system.

Other Chemicals

Pesticides do not represent health risk in case they are used under proper conditions. Maximum residue limits have been established for permitted pesticides and regulated by Environmental Protection Agency (EPA) (Rhodehamel, 1992).

1. Increase in the use of nitrate containing fertilizers has caused the accumulation of nitrates in some plants (Anon. 1997).
2. Antibiotics are mainly used for the treatment of infections in animals. However, they are sometimes used as growth stimulating agents also. They increase the chance for evolution of antibiotic resistant pathogens in human (Anon. 1997).
3. Maximum tolerable limits have been established for toxic elements including mercury, lead, arsenic and cadmium. Toxic elements as well as polychlorinated biphenyls (PCBs) contaminate foods as the result of environmental pollution (Jones, 1989).
4. Some chemicals such as polycyclic aromatic hydrocarbons (PAH) occur in foods during processing at high temperatures (>300^0C). Their concentrations increase with direct exposure to flame. They can also contaminate plants through accumulation of these compounds found in air (Shibamoto and Bjeldanes, 1993).

5. Detergent, disinfection residues and migration from food packaging materials are also considered among chemical hazards in foods. Control methods for chemical hazards are given in Table 2.9.

Table 2.7 Control methods for chemical hazards

Control before receipt
Raw materials specifications
Supplier guarantees
Random checks
Control before use
Check the purpose for the use of chemical
Control purity, formulation and labeling
Check the quantities to be used
Control storage and handling conditions
Control all the chemicals in facility
Review uses
Record of uses

Source: Rhodehamel (1992)

Physical Hazards

Foreign objects such as glass, wood, stones, metal, bone, plastic are the main physical hazards. They can cause injuries to man. Their presence in foods also indicates that foodstuffs were not processed under hygienic conditions.

Critical Control Points

Critical control points (CCPs) are any point or procedure in a specific food where loss of control may result in an acceptable health risk.

Hazards can be controlled during:

A. **Growing:**
   i. The use of antibiotics
   ii. Application of pesticides
   iii. Location of growing field

B. **Ingredient receiving:**
   i. Temperature control

C. **Processing:**
   i. Mixing of Ingredients
   ii. Thermal processing

D. **Distribution:**
   i. Time-temperature control

Critical Limits

Critical limits are minimum or maximum established values to control hazards at each CCPs. Exceeding critical limits indicates that the product may represent is a health hazard. Most frequently used critical limits are given below (Moberg, 1992).

- Time
- Temperature
- Humidity
- Moisture content
- pH
- Preservative
- Salt concentration
- Available chlorine

- Viscosity

Monitoring Critical Control Point Limits and Corrective Actions

Monitoring and record keeping are essential to HACCP system's success. Monitoring parameters can be qualitative and quantitative. Temperature checks, testing (Microbiological, chemical etc.), sensory and visual examinations are examples of monitoring procedures. To establish an effective monitoring procedure the questions of what, why, how, where, who and when must be answered (Hudak-Roos and Garret, 1992.

Corrective actions cover procedures that should be applied when a deviation occurred. Examples of corrective actions include continued cooking until 70^0C; addition of acid to reach target pH; addition of preservative to achieve proper concentration etc.

Record Keeping and Verification

Record keeping provides evidence of food safety, documentation for audit. In addition it is a tool for personnel training and solving problems (Stevenson and Hum, 1992).

Verification is the sum of activities other than monitoring that determines the validity and compliance with HACCP program (Prince, 1992).

HACCP is very beneficial to individual food processing industries and the world at large. It controls potential food safety risk in the manufacturing of food products. It helps to eliminate the risks involved in food production by identifying the main control points where risks lie to ensure a safe product at the end of the production process. In food processing operations, HACCP is important because it avoids hazards in an establishment by analyzing each component of the operation from delivery to service. Other benefits of HACCP include

- Saves business money in long run
- Avoids food poisoning
- Increases food safety standard
- Increases food quality standard
- Organizes process to produce safe food

2.8 HACCP of Fermented Food Condiments

The consumers' preference for safe and quality food products is increasing and the need to develop strategy to improve safety of condiments is essential for product competitiveness with standard bouillon cubes as well as guarantee product acceptability and consistency

(Oguntoyinbo, 2015). Hazard Analysis Critical Control Points is a systematic approach identification of hazard and prevention strategy for the improvement of food safety without necessary reliance on end product testing (Oguntoyinbo, 2015). Hazard analysis consists of evaluation of raw materials, water and its sources, environment, fermentation vessel, personal hygiene and other sources of contamination during processing as well as handling during packaging of final products (Oguntoyinbo, 2015; Rabi *et al.*, 2013). The raw seeds, sands used in dehulling, diluent, paw-paw leaves used as additives as well as final moulding, tabletting and display for sale to the consumers are Critical Control Points in the production of "dawadawa", a food condiment (Rabi *et al*., 2013) and these Critical Control Points are capable of contaminating the final products which could predispose the consumers of the products to food borne illnesses.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Procurement of Samples for Study

The samples, creeping melon seeds (*Citrullus vulgaris*) climbing melon seeds (*Cucumeropsis manni*), castor oil seeds (*Ricinus communis*) and fluted pumpkin seeds (*Telfairia occidentalis*) were bought at open market from different states of Nigeria. The choice of samples source was made because samples are not indigenous and not produced in sufficient quantities in the areas where they are mostly utilized. Also the climatic conditions and soil type could affect the composition of the samples. Different samples of ogiri produced from those substrates were collected from local (traditional) producers who were engaged to do so. Some of the ogiri samples were also bought at open markets.

3.1.1 Experimental Sites

The Microbiological analyses were carried out in the microbiology laboratory, Nnamdi Azikiwe University, Awka North Local Government Area while the physicochemical analyses were carried out in National Agency for Food and Drug Administration and Control (NAFDAC) zonal laboratory, Agulu in Anaocha Local Government Area both in Anambra State, Nigeria.

Plate 3.1 Various substrates for "ogiri" production

Key: 1 = Climbing melon seeds
2 & 3 = Creeping melon seeds
4 & 5 = Castor oil seeds
6 = Fluted pumpkin fruits
7 = Fluted pumpkin seeds

3.1.2 **Production of "Ogiri"**

The traditional method of processing 'ogiri' by Nzelu (2010) was modified to become appropriate for the study as shown in Figure 3.1 The mature seeds of castor oil, creeping melon, climbing melon and fluted pumpkin were sorted and aseptically dehulled. Ten wraps of 250g each were boiled for six hours. The boiled seeds were drained and left to ferment at room temperature. One wrap each was mashed in a disinfested mortar and the resultant paste, the 'ogiri' was finally wrapped in blanched plantain leaves (*Musa sapientum*).

Microbiological and chemical analysis of 'ogiri' from different substrates were carried out each day as fermentation proceeded. One gram of each sample was dissolved in 9ml of peptone water and diluted using a ten fold serial dilution prior to microbial analysis.

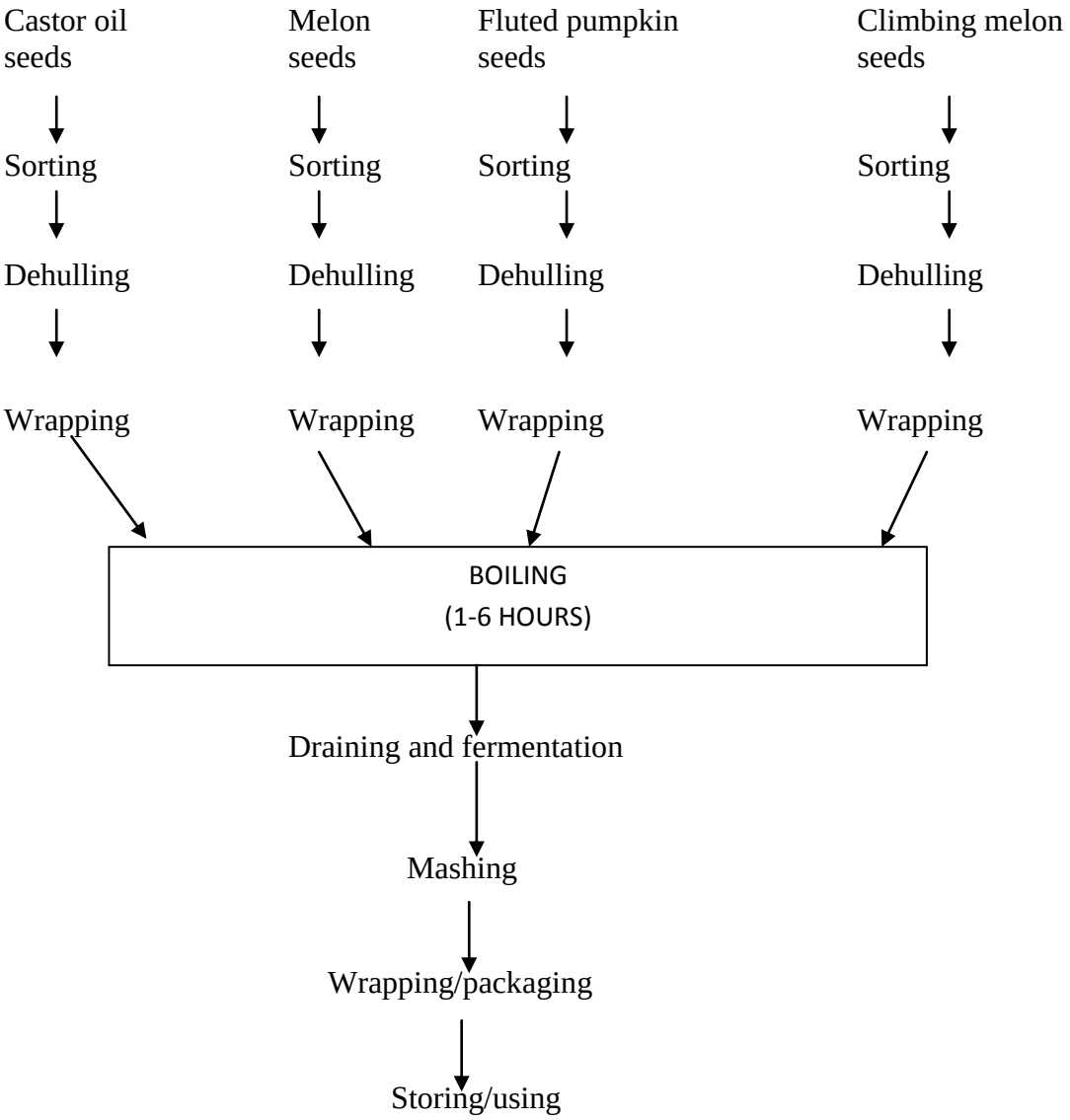


Fig 3.1: Process flow chart for the laboratory production of ogiri.

3.2 **Microbial Analysis of 'Ogiri' Samples**

3.2.1 **Total Viable Counts**

This was carried outby pour plate method as described by Tasie and Okafor (1999). One gram of each sample was weighed using electronic weighing balance (0106-1) and dissolved in 9ml of peptone water and diluted using a ten fold serial dilution. Zero point one millilistre of each sample suspension was taken from the dilutions 10^{-4}, 10^{-5} and 10^{-6} and inoculated on Nutrient agar, MacConkey agar, plate count agar, *Salmonella shigella* agar and sabouraud dextrose agar in duplicate and incubated at room temperature (maximum of 35^{o}C) for 24-72 hours. The mean of the replicate plating was calculated and the total viable count was calculated by the formular V= N/V x D expressed as cfu/g. Pure cultures of isolates were stored in agar slants in a refrigerator at 4^{o}C. The pure cultures were characterized by cultural, morphological and biochemical tests which included Gram staining, motility test, catalase test, coagulase test, citrate test, oxidase test, indole test, methyl red test, Voges-Proskaur test, nitrogen reduction test and sugar fermenation as described by Marvin (1984) and Duguid (1986) respectively. The isolations were made at material collection before sorting, during fermentation, after mashing and storing/using.

3.2 **Detection of Faecal Coliforms**

The methods of Tasie and Okafor (1999) was used. The test was carried in three stages.

Presumptive Test

One gram of each sample was suspended in 9ml of peptone water. Five tubes containing 5ml of single strength lactose broth and 1ml of samples were incubated at 35^{o}C for 24 hours with inverted durham tubes. Also the remaining 5 tubes with 5ml launy lactose broth and 0.1ml sample suspension was also incubated at 35^{o}C for 24 hours. The 10ml of sample suspension was also added to 5 tubes containing 10ml of double strength lactose broth.

Gas production indicated positive presumptive test while absence of gas production indicated negative presumptive test.

Confirmatory Test

A loopful of culture from the positive presumptive test was aseptically transferred to fresh tubes containing brilliant green lactose bile broth and incubated at 35^{o}C for 48 hours. Turbidity and production of gas indicated positive confirmatory test.

Completed Test

Cultures from positive confirmatory test was streaked on Eosin Methylene Blue (EMB) and inoculated at 35^{o}C for 24 – 48 hours. Presence of colonies with green metallic sheen differentiated *E. coli* from other coliforms.

3.2.2 Enumeration of Coliforms

The membrane filter technique was adopted. A sterile membraneabsorbent pad was placed in a Petri dish with sterile forceps and 2ml of the medium (teepol broth) added. Ten mililitress of the sample suspension was filtered using pre-sterilized membrane filter of 0.45μm pore size. The filter disc was transferred to nutrient pad previously prepared and incubated at 35^{o}C for 24 hours. The colonies were then counted.

3.3 Identification of Bacterial Isolates

The identification of bacterial isolates was confirmed using morphological, biochemical and physiological tests as described by (Dimejesi and Iheukwumere, 2014).

3.3.1 Motility Test

This was carried out using soft agar stabbing (tube method). Each isolate was transferred with sterile inoculating needle into a test tube containing nutrient broth. The tube was plugged with cotton wool and incubated for 24-48 hours. After 24-48 hours of incubation, a well dispersed growth from the line of inoculation indicated positive result.

3.3.2 Hydrogen Sulphide Test

Agar stabbing method (tube method) was also used. Each isolate was transferred with a sterile inoculating needle into a test tube containing nutrient broth and incubated for 24-48 hours.

Presence of black precipitate at the bottom of the test tube indicated production of hydrogen sulphide.

3.3.3 **Coagulase Test**

This was carried out using slide method. A loopful of distilled water was dropped on a clean slide. A small amount of a 24-hour old culture was mixed with the distilled water on the slide. The test suspension was treated with a drop of citrated plasma and mixed properly with the sterile inoculation loop. A control was also prepared without plasma.

Clumping of the organism (agglutination) within 5-10 seconds indicated positive test, while absence of agglutination showed negative result.

3.3.4 **Indole Test**

One and half grams of peptone water and 1.5g of glucose were dissolved in 150ml of water. The solution was dispensed into test tubes and autoclaved at 121^{o}C for 15 minutes. The medium was inoculated with the test-organism and incubated at 37^{o}C for 48 hours for optimum accumulation of indole. Zero point five million of Kovac's reagent (prepared by dissolving 10g of p-Dimethyl – aminobenzaldehyde in 150ml of amyl alcohol or 50ml of conc. HCL was added to each test tube and shaken gently. The development of red colour indicated positive reaction.

3.3.5 **Methyl red test**

One and half grams of peptone water and 1g of glucose were dissolved in 100ml of water, dispensed into test tubes and autoclaved at 121^{o}C for 15 minutes. The medium was inoculated with the test-organism and incubated at 37^{o}C for 48 hours. About 5 drops of methyl red indicator reagent was added in each test tube and mixed. Positive result was bright red while negative result (tests) was yellow.

3.3.6 **Voges-Proskauer Test**

One and half grams of peptone water and 1g of glucose were dissolved in 100ml of water, and dispensed into test tubes and autoclaved at121^{o}C for 15 minutes. The medium (glucose peptone water) was inoculated with the test-organism and incubated at 30^{o}C for 48 hours. Half a mililitre of O' Meara reagent (40g potassium hydroxide and 0.3g creatine in 100ml of diluted water) was added and mixed. A pink colour indicated positive reaction.

3.3.7 **Citrate Utilization Test**

Simmon's citrate medium, a modification of Koser's medium with agar was used. Agar was added to solidify the medium. This was dispensed in test tubes, autoclaved at 121^{o}C for 15

minutes and allowed to set as slopes (slants). The Simmon's medium consisted of Koser's medium (11g), agar 30g and bromothymol blue (40ml) as an indicator was added. The solidified medium was incubated at 37^{o}C for 96 hours. Blue colour and streak of growth indicated positive results.

3.3.8 **Urease Test**

About 2.4g of urea-based medium was dissolved in 95ml of distilled water and autoclaved at 121^{o}C for 15 minutes in a conical flask. Five mililitres of sterile urea was pipetted into each bijou bottle and also autoclaved. The urea agar based medium and the sterile urea were mixed at almost gelling temperature, solidified in slant and inoculated. These were incubated at 37^{o}C for 24-48 hours. Development of pink colour indicated positive urea test while no colour change indicated negative urea activity.

3.3.9 **Nitrate Reduction Test**

The test medium was prepared by dissolving 0.4g of potassium nitrate (KNO_3), and 10g of peptone water in 22ml of distilled water. About 5ml of the mixture was pipetted in different bijou bottles and autoclaved at 121^{o}C for 15 minutes. These were inoculated with a test organism and inoculated at 30^{o}C for 24-96 hours. The test reagents A and B were also prepared. The solution A $\rightarrow$ (8g of H_2SO_4 in 11ml of 5N acetic acid). Test solution B $\rightarrow$ (5g of x – naphthylamine in 11ml of 5N acetic acid).

After incubation, equal volumes of test reagent A and B were mixed and 0.1ml of the mixture was added in each bijou bottle. A red colour developing within a few minutes indicated positive reaction (i.e. presence of nitrite) while no colour change indicated negative reaction.

3.3.10 **Catalase Test**

Two drops of hydrogen peroxide was added to a smear of a 24-hour old culture of isolate on a glass slide. Evolution of gas as white froth indicated a positive reaction while absence of gas indicated negative reaction.

3.3.11 **Oxidase Test**

A strip of Whatman's No. 1 filter paper was soaked in a freshly prepared 1% solution of tetramethyl-p-phenylene diamine dihydrochloride, drained for 30 seconds, air-dried and

stored in a dark bottle which was gently sealed with screw cap. The dried paper which had a light purple tint was laid on a Petri dish and moistened with distilled water. The colony to be tested was smeared on the moist area. Development of an intense deep purple hue within 10-60 seconds was considered positive oxidase test while absence of colouration or colouration later than 60 seconds was considered oxidase reaction.

3.3.12 **Sugar Fermentation**

This was done to investigate the ability of the isolates to utilize different sugars. The following sugars were used: glucose, maltose, sucrose, lactose, manitol and sorbitol. One gram of each sugar was dissolved in 100ml of distilled water and dispensed in different test tubes. One and half grams of bacteriological peptone and 1ml of bromocresol purple were added to different test tubes (i.e. the mixture of each sugar, bacteriological peptone and bromocresol purple) were dispensed into test tubes and sterilized with inverted Durham tubes at 115^{o}C for 10 minutes. These were incubated for 24 hours at 37^{o}C. Change of colour from purple to yellow indicates positive test which is the production of acid. Presence of air bubbles at the top Durham tubes (empty space towards the bottom of the inverted Durham tubes) indicated gas production and absence of air bubbles indicated no gas production.

3.4 **Identification of Fungal Isolates**

This was carried out by wet mount and slide culture method as described by Tasie and Okafor (1999).

3.4.1 **Lactophenol Cotton Blue Stain**

The apparatus and the working surfaces were disinfected. The isolates were placed on the slides and mixed with 2 drops of lactophenol cotton blue stain and covered with cover slip sealed with nail hardner and viewed under microscope (x40 objective lens)

3.4.2 **Slide Culture**

This was done to observe the morphological characteristics of the isolates without disturbing the arrangement of spores and hyphae. A 100mm square agar block (SDA) each were placed on sterile slides. The slides were incubated with speck of fungal colony and aseptically covered with cover slip. The whole arrangement was placed on a sterile Petri-dishes containing small amount of sterile water and supported on a V-shaped sterile glass rod. The small amount of water helped to prevent the agar from drying out. The petridishes were

covered and incubated at 25-30^{o}C. Viable growth was examined at intervals when the viable growth was observed, the cover slips were removed and the lactophenol cotton blue mount was performed and examined microscopically for sporulation.

3.4.3 **Molecular identification of Bacterial and Fungal Isolates**

This was carried out by the method described by Centre for Agriculture and Bioscience International (CABI) and certified by Caine, T.S, Identification Operation Manager. All original samples were subjected to a purity check.

Bacterial samples were processed using partial 16S rDNA sequencing analysis.
Fungal samples were processed using ITS rDNA sequencing analysis.

All procedures were validated and processing undertaken in accordance with CABI's in-house methods as documented in TPs 61-68 and TP70 for bacteria and TPs 72-80 for filamentous fungi.

Molecular assays were carried out on each sample using nucleic acid as a template. A proprietary formulation [microLYSISR-PLUS (MPL), Microzone, UK] was subjected to the rapid heating and cooling of a thermal cycler, to lyse cells and release deoxyribonucleic acid (DNA).

Following DNA extraction, Polymerase Chain Reaction (PCR) was employed to amplify copies of the partial 16S fragment of rDNA in vitro for bacteria and the ITS fragment of rDNA in vitro for filamentous fungi.

The quality of the PCR product was assessed by undertaking gel electrophoresis.

PCR purification step was carried out to remove unutilized dNTOs, primer, polymerase and other PCR mixture compounds and obtain a highly purified DNA template for sequencing. This procedure also allowed concentration of low yield amplicons.

Sequencing reactions were undertaken using BigDye Terminator v3.1 kit from Applied Biosystems (Life Technologies, UK) which utilizes fluorescent labeling of the chain terminator ddNTPs, to permit sequencing.

Removal of excess unincorporated dye terminator was carried out to ensure a problem-free electrophoresis of fluorescently labeled sequencing reaction products on the capillary array AB 3130 Genetic Analyzer (DS1) DyeEx^{TM} 2.0 (Qiagen, UK)

Modules containing prehydrated gel-filtration resin were optimized for clean-up of sequencing reactions containing BigDye terminators. Dye removal was followed by suspension of the purified products in highly deionised formamide Hi-Di^{TM} (Life Technologies, UK) to prevent rapid sample evaporation and secondary structure formation.

Samples were loaded onto the AB 3130 Genetic Analyzer and sequencing undertaken to determine the order of the nucleotide bases, adenine, guanine, cytosine, and thymine in the DNA oligonucleotide.

Following sequencing, identification were undertaken by comparing the sequence obtained with those available in European Molecular Biology Laboratory (EMBL) via the European Bioinformatics Institute (EBI)

3.5 **Physicochemical Analysis**

3.5.1 **Determination of Protein**

Determination of protein was carried out by Kjeldahl's method as described by Egan *et al* (1981) and in NAFDAC Standard Operation (SOP) for protein determination. The analysis was carried out in three stages: Digestion, Distillation and Titration.

Digestion

Two grams of each sample was weighed out using electronic weighing balance (METLAR 200)and put in a 250ml Kjeldahl digestion flask. Then 10g of copper sulphate and 3g of sodium sulphate were added together and 3g of the mixture was added to the sample. Two pieces of Kjeldahl tablet was also used as alternative to mixture of copper sulphate and sodium sulphate. One gram of anti-bumping granules (chips) was added as well as 20ml of concentrated H_2SO_4. The flask and its content was placed in Kjeldahl's temperature regulated digester and digested for 3-4 hours as shown in Appendix 1, allowed to cool prior to distillation.

Distillation

Fifty mililitres of 10% sodium citrate solution was added to the digest in the flask. Then 10ml of 30% w/v of NaOH, two pieces of zinc pellets (granules) and 10ml of 2% boric acid were added in a 250ml conical flask. Also 1-2 drops of methyl red indicator solution was added to the mixture in the conical flask and placed under the mouth of the condenser. The distillate was then collected in the receiver flask and was used for titration.

Titration

Thirty mililitres of the distillate was put in a 250ml conical flask and titrated against 0.1M HCL to a purplish end point. The percentage nitrogen was calculated and then multiplied by a factor (6.25) to get the percentage protein.

$$\% \text{ Nitrogen} = \frac{\text{Titre} \times Ma \times 0.0140}{W} \times 100$$

where Ma = Molarity of acid used

W = weight of sample

Then % protein = % nitrogen x 6.25

3.5.2 **Determination of Free Fatty Acid**

This was carried out using Association of Official Analytical Chemist (AOAC, 2006) official method. Five gram of each sample was put in a conical flask and heated at 60-70^{o}C. Then 100ml of ethanol and 2ml phenolphthalein indicator were added to the heated sample and titrated against 0.25m NaOH solution to a faint purple colour that persisted for 20 minutes. Then the free fatty acid content was calculated as oleic acid and also as palmitic acid and expressed in mg/ml.

3.5.3 **Determination of Total Ash**

This was carried out by the method described in NAFDAC Standard Operation Procedure (SOP, 2014) for determination of total ash. For each sample, a clean dish (crucible) was weighted and the weight recorded as W_1. Then 5g of the sample was weighed directly into the dish and the weight (weight of the dish and sample) and recorded as W_2. The dish and the sample were charred over a flame in a fume cupboard until no smoke was given off. The crucible and the content were transferred into a muffle furnace (AAF 1100) at 550^{o}C-600^{o}C and left to ash. The fully ashed sample (black ash in colour) was cooled in a decicator and the weight recorded as W_3. The percentage ash was then calculated using the formulator.

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

3.5.4 **Determination of Moisture Content**

This was done using electronic moisture analyzer - Sartorius moisture analyzer (MA45). One gram of each sample was weighted using the in-built balance of the moisture analyzer. The start/on button was pressed. The reading was taken when signal for completed test (steady temperature of 105^{o}C was noted).

3.5.5 **Determination of Crude Fibre**

This was carried out by the method described in NAFDAC Standard Operation Procedure (SOP) for crude fibre determination. Two grams of the sample was put in a 250ml conical flask. Then 20ml petroleum ether and 200ml of 1.25% NaOH were added into the conical flask and boiled in a hot plate stirrer for 30 minutes. The residue was filtered out using Whatman's No. 1 filter paper. The weight of empty crucible was recorded as W_1. The filter paper and the residue was placed on the crucible dried in the furnace and the weight of the crucible together with that of residue was recorded as W_2. Then the loss of weight of samples after ashing was taken as crude fibre content

$$\% \text{ crude fibre} = \frac{W_3 - W_6}{\text{Wt of samples}} \times 100$$

Where W_3 = weight of residue

W_6 = Wt of ash = $W_5 - W_4$

W_5 = Wt of crucible + ash

W_4 = Wt of empty crucible

3.5.6 **Determination of total Carbohydrate**

Total carbohydrate was obtained by different using

Total carbohydrate = 100 - (% moisture + ash + protein + fat)

3.5.7 **Determination of Volatile Acidity**

This was carried out by the method (Dimejesi and Iheukwumere, 2014). The distillation apparatus set up as shown in appendix 3.Five grams of each sample was dissolved in 100ml of distilled water and poured into round bottom flask and also 1g of anti-bumping chips added.

Then lL (1000ml) of water was poured into the steam developer (vapour developer) vessels. Ten millilitres of distilled water was poured in the receptor flask meant for collecting the distillate. Sixty mililitre of the distillate was poured into a 250ml conical flask, heated to boil, 1ml of 1% alcoholic phenolphthalein indicator solution was added and titrated with 0.81M NaOH to a faint pink colour and the volume recorded as 'a' ml. Five mililitres of 25% H_2SO_4 and 0.1mle of 1% starch solution was also added and immediately titrated with 0.2M iodine solution in the burette to a blue colour lasting for 10 minutes and the volume recorded as 'b' ml. Then the volatile acid content was calculated as acetic acid in mg/l using the formula 0-12 (a-2b)

Where ‘a’ = volume of NaOH used

‘b’ = volume of iodine used

3.5.8 **Determination of Titrable Acidity**

The method of Amerine *et al* (1980) was used. Two hundred mililitres of distilled water was poured into a 250ml conical flask and brought to boil. Then 2 drops of 1% aqueous phenolphthalein indicator was added to the boiling water in the conical flask and titrated against 0.1M NaOH to a faint pink colour and the volume recorded as V_1. Five mililitres of the sample suspension was added and titrated against 0.1M NaOH to a faint pink colour and the volume recorded as V_2. The titrable acidity was calculated and expressed as tartaric acid in g/100ml using the formula.

$$\text{Titrable Acidity g/100ml} = \frac{V_1 \times MV \times 75 \times 100}{V_2 \times 1000}$$

Where V_1 = Volume of NaOH for neutralizing the water

V_2 = Volume of NaOH for neutralizing the sample

M = Molarity of NaOH

V = Volume of sample used

75 = Equivalent weight of tartaric acid

3.5.9 **Determination of pH**

The pH values of the samples were determined with pH meter (JENWAY 3510). The pH meter was standardized with buffers of pH 4 and pH 8 respectively and then with distilled water. The electrode was then inserted in each sample suspension and the readings were taken.

3.6 **Determination of Total Afflatoxin**

Samples were extracted and the extracts analyzed using Enzyme Linked Immuno Sorbent Assay (ELISA) machine (Statfax 303). The extraction was carried out by the method described in the NAFDAC Standard Operation Procedure (SOP) for Mycotoxin analysis. Five grams of each sample was put in a conical flask and 25ml of tween ether and ethanol was added in the ratio of 30:70 (3:7). The mixture in the conical flask was put in orbital shaker (DS 25002) set at 125 rpm for 20-30 minutes to aid homogenization. The sample was then filtered with Whatman No. 1 filter paper and the filterate was used for Mycotoxin analysis.

Mycotoxin Analysis was carried out using Agra Quant Aflatoxin (1-20 Ppb) Test kit. Afflatoxin conjugate was pipetted into the desired number of wells using multi-channel pipette set at 200 µl. The standard was pipetted in the ascending order from 0.00 – 20ppb to the first well using the multi-channel pipette set at 100µl. Using the same multi-channel pipette set at 100 µl, the filtered samples were pipetted into the well. The standard was equally prepared. The whole arrangement was incubated for 15 minutes on a bench and blue colour change was observed. Finally, stop solution was added in all the wells using multi-channel pipette set at 100 µl and colour change from blue to yellow was observed. The wells were set well and inserted into the ELISA (Stat fax 3030) machine where the result was produced and interpreted.

3.7 **Determination of Heavy Metals**

3.7.1 **Determination of Cadmium**

This was determined by the use of smart electrophotometer (Lamotte). Cadmium was selected from the menu button. The samples were extracted using soxhlet extraction apparatus (GT 301). Ten mililitres of the extracted sample were poured into a cuvette (tube). The tube was inserted into the spectrophotometer chamber and scanned as blank. The tube was removed from the spectophotometer. The 1ml of buffered ammonium reagent (402t), 2 drops of 10% sodium citrate, 0.5ml of PAN indicator (4021) cap and 0.5ml stabilizing reagent (4022) cap were added into the test tube, mixed together and inserted into the spectophotometer chamber. Scan sample was selected from the menu and the result recorded in ppm.

3.7.2 **Determination of Lead**

This was determined using smart spectrophotometer. The tube to be used was rinsed with test sample and filled with 10ml sample (extract) and scanned as blank. The tube was removed from the spectrophotometer. Then 5ml of ammonium chloride buffer (4032), 3 drops of 10% sodium cyanide, 0.5m Pyridylazo resorcinol (PAR) indicator and 0.5ml of stabilizing reagent were also added to the tube, mixed and inserted into the chamber. The menu scan sample was selected and the result recorded in ppm.

Thirty three copper Unit Dose Vials (UDV) was selected from the menu. The tube was rinsed with test sample water and filled with 3ml of the test sample and scanned as blank. The tube was removed from the chamber. Then 3ml of sample was also added to a copper

UDV (4314) and allowed to stand for two minutes. The tube was inverted severally to mix and scan sample menu was selected and the result recorded in ppm.

3.7.3 **Determination of Iron**

This was determined using smart spectrophotometer. The tube was rinsed with sample water and filled back with 10ml sample. The tube containing the sample was inserted into the chamber and scanned as blank. The tube was removed from the chamber and six (6) drops of Acid phenanthiolene indicator (2776) cap were added. The tube was inverted several times to mix the reagents and allowed to stand for five minutes for colour development. The tube was inserted into the chamber and the menu, scroll sample was chosen and the total ion content was read off and recorded in ppm.

3.7.4 **Determination of Calcium and Magnesium**

This was carried out by Unit Dose Vials (UDV) method using smart spectrophotometer. The menu thirteen (13) Calcium and Magnesium Hard – UDV was chosen. The vial was rinsed with sample water. Three (3) mililitres of the sample was added to the vial with syringe (1184). The vial was inserted into the chamber and the menu scan blank was chosen. The vial was removed from the chamber of the spectrophotometer. Then 3ml of sample to calcium hardness UDV vial (4309) and shaken vigorously for 10 seconds. The tube was inserted into the chamber and the menu, scan sample was chosen and the total hardness recorded.

Concentration of calcium was calculated using

$$Ca = \frac{\text{Total hardness} \times 2}{3}$$

while the magnesium content was calculated using

$$\frac{Ca}{3}$$

where Ca = concentration of Calcium

3.8 **Determination of Amino Acid Profile**

The Amino Acid profile in each sample was determined using methods described by Benitez (1989). Each sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon sequential Multi-Sample Amino Acid Analyzer (TSM).

3.8.1 **Defatting of Sample**

The sample was defatted using chloroform/methanol mixture of ratio 2:1. About 4g of the sample was put in extraction thimble and extracted for 15 hours in Soxhlet extraction apparatus (AOAC, 2006).

3.8.2 **Nitrogen Determination**

A small amount (200g) of ground sample was weighed, wrapped in Whatman filter (No. 1) and put in the Kjeldhal digestion flask. Concentrated sulphuric acid (10ml) was added. Catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added.

The flask and its content was heated for 3 hours in a Kjeldahl digestion apparatus until the colour turned green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (10ml) of the diluted solution with 10ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until 70ml of distillate was collected.

The distillate was then titrated with standardized 0.01 N hydrochloric acid to grey coloured. Percentage Nitrogen $= \frac{(a-b) \times 0.01 \times 14 \times V \times 100}{W \times C}$

3.9 **Determination of Critical Control Points**

This was determined using the decision tree as described by Rabi *et al*, 2013.

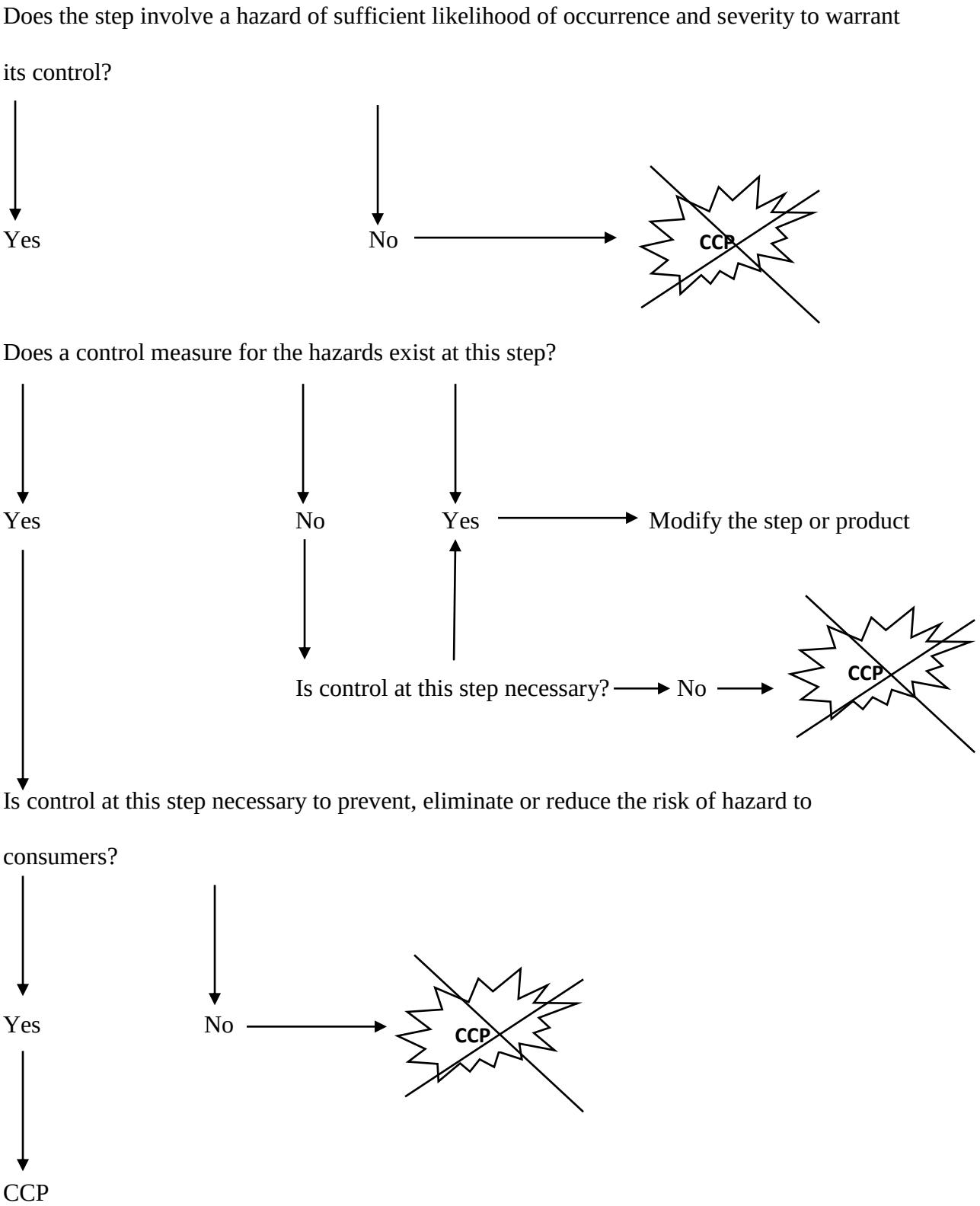


Fig. 3.2 Critical Control Point Decision Tree

Source: (Rabi *et al.*, 2013)

3.10 **Statistical Analyses**

The data obtained from the study was subjected to Analysis of Variance (ANOVA) using computer SPSS (2015) version 20. Differences in means were evaluated at 5% (0.05) level of significance and direction of interaction between treatment using Turkey HSD POST HOC Test.

CHAPTER FOUR

4.0 RESULTS

The mean heterotrophic bacterial counts were high in virtually all the samples. However, the highest mean heterotrophic bacterial count was observed in "ogiri" produced from castor oil seed (5.6×10^8cfu/g) followed by creeping melon (4.8×10^8cfu/g), fluted pumpkin (3.7×10^8cfu/g) and climbing melon seeds (2.7×10^8cfu/g) (Table 4.1).

The "ogiri" produced from fluted pumpkin showed the highest mean coliform count (2.5×10^7cfu/g) followed by climbing melon (2.4×10^7cfu/g), castor oil seed (2.3×10^7cfu/g) and creeping melon (1.6×10^7cfu/g) as shown in Table 4.2.

The "ogiri" produced from castor oil seed showed the highest mean heterotrophic fungal counts (4.1×10^7cfu/g) followed by fluted pumpkin (3.2×10^7cfu/g), climbing melon (2.9×10^7cfu/g) and creeping melon (2.2×10^7cfu/g) as shown in Table 4.3

The "ogiri" produced from castor oil seeds recorded higher *Salmonella – Shigella* counts (3.6×10^7cfu/g) than its counterpart samples, climbing melon (3.4×10^7cfu/g), fluted pumpkin (3.2×10^7cfu/g) and creeping melon seeds (2.7×10^7cfu/g). Table 4.4 shows mean *Salmonella – Shigella* Count of "ogiri" produced from creeping melon, castor oil, fluted pumpkin and climbing melon seeds.

Table 4.1 Mean Heterotrophic Bacterial Counts of "ogiri" Samples Produced from Creeping Melon, Climbing Melon, Fluted Pumpkin and Castor Oil seeds

Sample	**Mean Heterotrophic Count (cfu/g)**	
	Locally processed ($x10^8$)	**Laboratory Processed ($x10^6$)**
Creeping melon	4.8±0.1	1.1±0.2
Castor oil	5.6±0.3	0.5±0.2
Fluted pumpkin	3.7±0.2	1.8±0.1
Climbing melon	2.7±0.7	1.0±0.3

Table 4.2 Mean Coliform Counts of "ogiri" Samples Produced from Creeping Melon, Castor Oil, Fluted Pumpkin and Climbing Melon Seeds.

Sample	**Mean Coliform Count (cfu/g)**	
	Locally Processed ($x10^7$)	**Laboratory Processed ($x10^2$)**
Creeping melon	1.6±0.1	0.1±0.02
Castor oil	2.3±0.1	1.0±0.2
Fluted pumpkin	2.5±0.1	0.2±0.01
Climbing melon	2.4±0.1	1.0±0.01

Table 4.3 Mean Heterotrophic Fungal Counts of "ogiri" Samples Produced from Creeping Melon, Climbing Melon, Fluted Pumpkin and Castor Oil seeds

Sample	**Mean Heterotrophic Count (cfu/g)**	
	Locally Processed ($x10^7$)	**Laboratory Processed ($x10^6$)**
Creeping melon	2.2±0.001	0.4±0.001
Castor oil	4.1±0.001	0.3±0.001
Fluted pumpkin	3.2±0.1	0.5±0.01
Climbing melon	2.9±0.01	0.4±0.001

Table 4.4 Mean *Salmonella – Shigella* Counts of "ogiri" Produced from Creeping Melon, Castor Oil, Fluted Pumpkin and Climbing Melon Seeds.

Sample	**Mean *Salmonella-Shigella* Count (cfu/g)**	
	Locally Processed ($x10^7$)	**Laboratory Processed**
Creeping melon	2.7±0.01	NG
Castor oil	3.6±0.1	NG
Fluted pumpkin	3.2±0.1	NG
Climbing melon	3.4±0.1	NG

Key NG = No Growth

Table 4.5 shows the mean viable counts of unprocessed and processed "ogiri" samples from climbing melon, castor oil, creeping melon and fluted pumpkin seeds. The mean viable counts were higher in the processed samples than the unprocessed samples with castor oil seed recording the highest mean viable count in processed product (5.2×10^8cfu/g) followed by creeping melon (4.7×10^8cfu/g), fluted pumpkin (4.5×10^8cfu/g) and climbing melon (3.7×10^8cfu/g). In the unprocessed samples, creeping melon recorded the highest mean viable count (9.8×10^4cfu/g) followed by castor oil seeds (9.5×10^4cfu/g), climbing melon (8.3×10^4cfu/g) and fluted pumpkin (7.6 x 104cfu/g).

Table 4.6 shows the mean viable count of utensils, packaging materials, water and handlers involved in "ogiri" production. The mean viable bacterial count was higher in mortar and pestle (4.5×10^6cfu/ml) than its counterpart samples, leaves (2.5×10^6cfu/ml), water (1.2×10^6cfu/ml), nasal swab (5.0×10^3cfu/ml), string (3.8×10^3cfu/ml) and skin swab (3.4×10^3cfu/ml). There was higher coliform count in the leaves (2.5×10^6cfu/ml) than other samples – mortar and pestle (2.4×10^6cfu/ml), water (2.1×10^6cfu/ml), skin swab (3.3×10^3cfu/ml), skin swab (2.8×10^3cfu/ml), and string (1.2×10^4cfu/ml). The leaves, mortar and pestle recorded the highest mean viable fungal count (2.0×10^4cfu/ml each) followed by water (1.0×10^4cfu/ml), nasal swab (4.0×10^2cfu/ml), skin swab and string (2.0×10^2cfu/ml each).

Table 4.5 Mean Viable Counts of Unprocessed and Processed Samples.

Sample	**Mean Heterotrophic Count (cfu/g)**	
	Unprocessed (Seeds) ($x10^4$)	**Processed (Ogiri) ($x10^8$)**
Creeping melon	8.3±0.05	3.7±0.10
Castor oil	9.5±0.03	5.2±0.04
Fluted pumpkin	9.8±0.1	4.7±0.02
Climbing melon	7.6±0.1	4.5±0.2

Table 4.6 Mean Viable Counts of the Utensils, Packaging Materials, Water and Handlers involved in "ogiri" Production.

Sample	**Mean Viable Count (cfu/ml)**			
	NA	**MA**	**SDA**	**SSA**
Leaves for wrapping	2.5×10^6	2.5×10^6	2.0×10^4	4.4×10^5
Water for mixing	1.2×10^6	2.1×10^6	1.0×10^4	2.0×10^4
Mortar and Pestle	4.5×10^6	2.4×10^6	2.0×10^4	6.5×10^6
Handlers (Nasal Swab)	5.0×10^3	2.8×10^3	4.0×10^2	NG
Handlers (Skin Swab)	3.4×10^3	3.3×10^3	2.0×10^2	Ng
String	3.8×10^3	1.2×10^4	2.0×10^2	1.0×10^4

Key: SSA = *Salmonella-Shigella*Agar

NG = No Growth

NA = Nutrient Agar

MA = MacConkey Agar

SDA = Sabouraud Dextrose Agar

A total of seventeen bacterial and four fungal genera were isolated. The isolates were characterized based on their cultural, morphological and biochemical properties using the Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994), the isolates were tentatively identified as *Pseudomonas*, *Staphylococcus aureus*, *Lactobacillus*, *Streptococcus*, *Proteus*, *Escherichia*, *Klebsiella*, *Salmonella*, *Shigella*, *Bacillus*, *Flavobacterium*, *Micrococcus*, *Actinomyces*, *Vibrio* and *Pediococcus* species. Also four types of fungal species were also isolated. The fungal isolates were characterized based on cultural and morphological propertiesaccording to (Laron, 2014 and Barneth and Hunter, 2000). The isolates were identified as *Aspergillus, Mucor, Penicillium* and *Trichoderma* species.

Eight out of the isolates were characterized and identified using 165 rDNA sequencing analysis for bacteria and ITS rDNA sequencing for fungi and the isolates identified as *Pseudomonasplecoglossicida*, *Bacillus fusiformis* (*Lysinibacillus fusiformis*), *Klebsiella pneumoniae*, *Enterobacter clocae*, *Leclercia adecarboxylata*, *Aspergillus fumigatus*, *Aspergillus terreus* and *Trichoderma reesei*, respectively as shown in Tables 4.7 and 4.8.

Table 4.7 Characteristics of Bacterial Isolates

Isolates	Cultural Morphology	Microscopic Morphology	Gram Reaction	Catalase	Citrate	Coagulase	Oxidase	Methyl Red	Nitrate	Indole	Voges Proskauer	Urease	H_2S	Motilitty	Lactose	Maltose	Glucose	Sucrose	Xylose	Manitol	Sorbitol	Probable organism
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
1	On nutrient and MacConkey agar, colonies are large, low convex, rough and oval in shape. Some are irregularly round about 2-3mm in diameter and emit fruity odour and also pigmented (green-yellow, blue-green	Straight and slightly curved rods	-	+	-	-	+	-	+	-	-	+	+	+	-	-	A	-	-	-	-	*Pseudomonas plecoglossicida*
2	Colonies are yellowish, moist and have smooth glistering surface on nutrient agar, appears pinkish on MacConkey agar and about 1-2mm in size.	Cocci in grape-like cluster with some singe and paired	+	+	-	+	-	+	+	-	+	+	-	-	A	+	A	A	+ A	A	A	*Staphylococcus* spp
3	Colonies are round, entire, low convex, smooth, transluscent, colourless and about 2-3mm in diameter on MacConkey agar	Slender irregular rods	+	-	-	-	-	-	-	-	-	-	-	-	+ A	+ A	+ A	-	A	A	A	*Lactobacillus* spp

4	Low convex discrete colonies about 0.5-1.0mm in diameter	Spherical cocci in short chains	+	-	-	-	+	-	-	-	-	+	-	-	+ A	+ A	+ A	+	A	-	-	*Streptococcus* spp
5	Very large swarming (spreading) growth (surface), emit putrefactive fishy odour and creamy in colour about 3-5mm in diameter	Coccobacilli in short chain and some are in pairs	-	+	-	-	-	+	+	+	-	+	+	+	-	-	A	A	A	-	-	*Proteus* spp
6	Colourless to greyish smooth colonies on nutrient agar, rose pink, large colonies of MacConkey agar about 2-3mm in diameter	Rod shaped	-	+	-	-	-	+	+	+	-	-	+	+	+ A	A	+ A	+ -	A	+ A	-	*Escherichia coli*
7	On MacConkey agar, colonies appear large, mucoid and red, colourless to grey on nutrient agar.	Rod shaped	-	+	+	-	-	-	+	-	+	+	-	-	+ A	A	+ A	+ A	A	+ A	A	*K. pneumoniae*
8	Colonies are greyish to white circular, moist, convex and transluscent in nutrient agar Pale yellow on MacConkey agar, colourless with black centre on SSA, about 2-3mm in diameter	Rod shaped	-	+	+	-	-	+	+	-	-	-	+	+	+ A	+ A	+ A	A	+ A	+ A	A	*Salmonella* spp

9	Smooth greyish colour, transluscent colonies on nutrient agar, colourles on MacConkey agar, colourless without blackening on SSA	Short rods in pairs	-	+	-	-	-	+	+	-	-	-	-	-	+ A	+ A	+ A	+ A	+ A	+ A	+ A	*Shigella* spp
10	Large, greenish, raised, wide-spreading with irregular fingerlike edges and opaque about 2-3mm in diameter	Long straight rods in single, some in pairs	+	+	+	-	+	-	+ -	-	+	-	-	+	-	+ A	+ A	+ A	A	A	A	*Bacilus Fusiforms*
11	Small, smooth, yellow and transluscent colonies about 1-2mm in diameter	Short slender rods	-	+	+	-	+	-	-	-	-	-	-	-	+ A	+ A	+ A	+ A	+ A	+ A	+ A	*Flavobacterium* spp
12	Smooth yellow colonies, shinny, raised and some with elevated centres, about 1-2mm diameter	Small irregular rods in pairs and tetrads	+	+	-	-	-	-	-	-	+	+	-	+	-	A	-	+ -	A	-	-	*Micrococcus* spp
13	White to yellow colonies that are star-shaped with irregular edges	Fetted mass of branding filament which are interlaced.	+	-	-	-	+	+	+	-	-	+ -	-	A	A	A	A	A	A	A	A	*Actinomyces* spp
14	Round , greenish to bluish colonies about 2-3mm in diameter	Curved and straight	-	+	+	-	+	+	+	+	+	+	-	+	-	A	A	-	A	A	A	*Vibrio*

15	Very small flat colonies, white to colourless colonies on nutrient agar, about 1mm in diameter.	Cocci in tetrad or in short chain	+	-	-	-	-	+	+	-	-	-	+	-	A	+ A	A	A	A	A	A	*Pediococcus* spp
16	Greyish smooth colonies	Gram negative Rods	-	+	-	-	-	+	+	+	-	+	+	+	-	-	A G	A G	-	-	A G	*Leclercia Adecarboxylata*
17	Large mucoid colonies on red Mackonkey agar	Rod shaped	-	+	+	-	-	-	+	+	-	+	+	-	-	+	A G	A G	A G	A G	A	*Enterobacter Cloacae*

Table 4.8 Characteristics and Identity of Fungi Isolates

Isolate	**Colony Morphology**	**Microscopic Morphology**	**Probable Identity**
1	Greenish white, Flat, irregular shaped, dry and dull	Septate, hyphae, conidia arranged like mob-heead	*Penicillium* spp
2	White filamentous colonies	Non-septate hyphae, spores enclosed in a sporangium	*Mucor* spp
3	Granular to wooly colonies that have some shade of yellow or yellow-brown	Long conidiophores	*Aspergillus* spp
4	1-2 concentric rings with green conidal production which is denser in the centre with irregular yellow zones	Conidia shape glubose to subglobose	*Trichoderma reesei*

Table 4.9 shows the total bacterial counts during the 6-day fermentation of castor oil, creeping melon, climbing melon and fluted pumpkin seeds for “ogiri” production. There was a general increase in the bacterial counts in the first 4 days of fermentation. The bacterial count in castor oil decreases on the fifth day and increased thereafter, while in the other samples (creeping melon, fluted pumpkin and climbing melon) there was a continuous decrease in the bacterial counts.

Table 4.10 shows total fungal count during the 6-day fermentation of castor oil, creeping melon, fluted pumpkin and climbing melon seeds for “ogiri” production. There was a general decrease in the fungal counts in all the samples with days of fermentation. However, the fungi disappear after 2-3 days.

Table 4.9 Total Bacterial Count during the Six-Day Fermentation of Castor Oil, Creeping Melon, Climbing Melon and Fluted Pumpkin Seeds for "ogiri" Production

Day	**Total Bacterial Count (cfu/g)**			
	Castor Oil Seed ($x10^9$)	**Creeping Melon Seed ($x10^6$)**	**Fluted Pumpkin Seed ($x10^6$)**	**Climbing Melon Seed ($x10^6$)**
0	0.9	0.8	0.6	0.7
1	1.0	1.2	2.0	1.1
2	1.2	1.3	3.1	0.5
3	1.4	1.6	3.3	0.4
4	1.6	1.9	3.5	0.3
5	1.4	1.6	2.2	0.2
6	1.9	1.3	1.5	0.1

Table 4.10 Total Fungi Count during the Six-Day Fermentation of Castor Oil, Creeping Melon, Fluted Pumpkin and Climbing Melon Seeds for "ogiri" Production.

Day	**Total Fungal Count (cfu/g)**			
	Castor Oil Seed ($x10^4$)	**Creeping Melon Seed ($x10^6$)**	**Fluted Pumpkin Seed ($x10^6$)**	**Climbing Melon Seed ($x10^6$)**
0	0.4	0.5	0.6	0.5
1	0.2	0.3	0.5	0.4
2	0.1	0.2	0.5	0.3
3	0.1	0.1	0.4	NG
4	NG	NG	NG	NG
5	NG	NG	NG	NG
6	NG	NG	NG	NG

Key: NG = No Growth

The result of proximate analysis of the unprocessed creeping melon, climbing melon, fluted pumpkin and castor oil seed samples and their resultant fermented products (“ogiri”) are shown in Table 4.11. The moisture content of the fermented products was higher (40.34-73.79%) than in the unprocessed substrates (seeds) (9.73-41.34%). The crude fibre contents of the fermented products were lower (2.0-3.0%) than the unprocessed seeds (2.5-3.5%).

The fat content decreased in all the samples from the unprocessed seeds (31.22-34.82%) to (22.00-25.20%) in the fermented products. The protein content was higher in the unprocessed seeds (8.40-13.48%) than in the fermented products (4.80-7.96%). The free fatty acid (FFA) content was higher in the fermented products (7.68-17-68%) than in the unprocessed seeds (5.25-16.64%). The carbohydrate content was higher in the fermented products (55.020-56.88%) than in the unprocessed seeds (46.51-48.33%).

The titrable acidity was higher in the fermented products (0.62-0.75g/100ml) than in the unprocessed seed (0.25-0.3g/100ml). The ash content is lower in the fermented samples (2.6-3.2%) than in unprocessed seeds (3.4-3.6%).

The volatile acidity was higher in the fermented products (0.07-0.08g/100ml) than in the unprocessed (0.03-0.05g/100ml) in virtually all the samples as fermentation progressed.

The pH in the unfermented seeds ranged from 6.2-6.4 and 7.2-7.5 in the fermented products

Table 4.11 Proximate Composition of Unprocessed and Processed Castor Oil, Fluted Pumpkin, *Cucumeropsis* and Melon Seeds

	Castor Oil Seed		***Cucumeropsis manii***		**Fluted Pumpkin Seed**		**Melon Seed**	
	Unprocessed	**Fermented**	**Unprocessed**	**Fermented**	**Unprocessed**	**Fermented**	**Unprocessed**	**Fermented**
Protein (%)	4.30±0.10	3.90±0.10	5.70±0.27	4.80±0.27	8.40±0.10	4.20±0.10	13.48±0.01	7.96±0.01
Fat (%)	34.82±0.01	25.20±0.10	31.22±0.01	22.00±1.00	15.60±0.10	5.00±1.00	23.49±0.01	17.40±0.10
FFA (mg/ml)	16.64±0.01	17.68±0.02	3.58±0.01	4.50±0.10	3.46±0.01	7.68±0.03	5.25±0.01	6.91±0.01
Ash (%)	3.0±1.00	3.2±0.17	0.2±0.10	0.15±0.01	3.40±0.10	1.60±0.10	3.60±0.10	2.60±0.10
Moisture content (%)	4.86±0.03	40.34±0.01	6.00±1.00	20.65±0.01	41.34±0.01	73.79±0.01	9.73±0.01	25.53±0.01
Crude fibre (%)	2.50±0.10	2.00±1.00	0.50±0.10	0.40±0.10	1.60±0.20	1.50±0.10	3.50±0.10	3.30±0.10
Carbohydrates (%)	46.51±0.03	55.02±0.01	48.33±0.03	56.88±0.01	31.26±0.01	38.70±0.01	36.51±0.02	44.99±0.01
Titrable acidity (g/100ml)	0.25±0.01	0.62±0.01	0.30±0.01	0.60±0.01	0.15±0.01	0.40±0.10	0.30±0.01	0.75±0.01
Volatile acidity (g/100ml)	0.05±0.01	0.08±0.01	0.04±0.01	0.07±0.01	0.03±0.01	0.06±0.01	0.02±0.01	0.04±0.01
pH	6.4±0.10	7.2±0.17	6.2±0.10	7.5±0.17	6.1±0.10	7.1±0.10	6.2±0.10	6.90±0.10

The results of the mineral analysis is shown in Table 4.12.

The calcium content was higher in the unprocessed seeds (20.00±1.00), 21.30±1.00 in climbing melon, than the fermented seeds of castor oil and *Cucumeropsis manii* (20.00±1 and 18.75±0.17) while is higher in the fermented fluted pumpkin and creeping melon seed (38.70-72.70ppm) as against that in unprocessed seeds (21.30-34.70ppm). The copper content is higher in all the unprocessed seeds except that of fluted pumpkin. There is higher concentration of cadmium in the unprocessed seeds (0.63-0.67ppm) than in the fermented products (0.40-0.60ppm). There was a decrease in the concentration of lead in virtually all the samples from unprocessed to fermented. The iron content is higher in the fermented products than in the unprocessed seeds except in that of *Cucumeropsis manii*. The magnesium content of unprocessed seeds are higher (11.6-15.3ppm) than in the fermented products (9.6-14.3ppm)

Table 4.12 Mineral Content of Unprocessed and Fermented Castor Oil, Fluted Pumpkin, *Cucumeropsis manii* and Melon Seeds

	Castor Oil Seed		***Cucumeropsis manii***		**Fluted Pumpkin Seeds**		**Melon Seed**	
	Unprocessed	**Fermented**	**Unprocessed**	**Fermented**	**Unprocessed**	**Fermented**	**Unprocessed**	**Fermented**
Calcium (ppm)	26.00±1.00	20.00±1.00	21.30±0.17	18.75±0.01	46.00±1.00	72.70±0.17	34.20±0.10	38.70±0.17
Copper (ppm)	0.33±0.02	0.18±0.01	0.38±0.02	0.27±0.03	0.23±0.02	0.26±0.01	0.98±0.01	0.60±0.10
Cadmiun (ppm)	0.63±0.02	0.60±0.10	0.59±0.01	0.40±0.22	0.20±0.10	0.10±0.02	0.67±0.01	0.10±0.01
Lead (ppm)	0.20±0.01	0.01±0.01	2.80±0.10	1.20±0.10	0.20±0.01	0.10±0.01	0.10±0.053	0.10±0.56
Iron (ppm)	0.50±0.10	0.80±0.10	0.89±0.01	0.70±0.01	1.42±0.05	0.53±0.01	1.35±0.01	0.52±0.01
Magnesium (ppm)	9.30±0.10	0.10±0.10	7.10±0.17	6.25±0.10	15.30±0.10	14.30±0.17	11.66±0.01	9.60±0.10

The amino acid analysis for various samples is shown in Table 4.13. There was a decrease in the concentration of amino acids from nearly all the unprocessed to the fermented samples, except in Argmine, Isoleucine and Leucine where the reverse is the case. The concentration of amino acid, proline remained the same (2.90g/100ml), Glutamic acid recorded the highest concentration of amino acid in both fermented and unprocessed samples (12.49-15.21g/100ml protein) followed by Aspartic acid, Argmine and Valine. The tryptophan contents were low (0.8-1.32g/100g protein) in virtually all the samples. Similarly, the concentrations of cystine and methionme were also low - (1.29-1.5g/100 protein) and (1.31-1.5g/100g protein) respectively in all the samples.

Table 4.13 Amino Acid Profile of the Samples

Concentration of amino acid (g/100g) Protein

Amino Acid	**Unprocessed Castor oil Seed**	**Fermented Castor oil Seed**	**Unprocessed Melon Seed**	**Fermented Melon Seed**	**Unprocessed *Cucumeropsis***	**Fermented *Cucumeropsis***	**Unprocessed Pumpkin Seed**	**Fermented Pumpkin Seed**
Lysine	3.79±0.01	3.49±0.01	3.60±0.20	3.71±0.01	4.20±0.01	4.10±0.01	3.30±0.20	3.10±0.01
Histidine	3.02±0.01	2.83±0.01	2.95 ±0.10	3.01±0.01	2.89±0.01	2.56±0.01	2.50±0.10	2.20±0.01
Arginnie	7.83±0.01	8.01±0.01	8.59±0.01	7.49±0.01	9.28±0.01	8.30±0.01	8.76±0.01	7.92±0.01
Aspartic acid	8.33±0.02	7.64±0.01	7.83±0.01	3.01±0.01	8.65±0.02	6.50±0.01	7.89±0.01	6.85±0.01
Threonine	3.15±0.02	2.49±0.01	3.07±0.01	2.72±0.02	2.30±0.02	2.12±0.01	2.50±0.01	2.30±0.02
Serine	3.49±0.02	3.11±0.01	3.70±0.01	3.52±0.01	4.23±0.02	4.05±0.01	3.55±0.01	3.16±0.01
Glutamic acid	13.43±0.01	12.93±0.01	14.08±0.01	13.22±0.10	15.21±0.01	14.21±0.01	13.58±0.01	13.11±0.10
Proline	2.90±0.10	2.90±0.10	3.13±0.01	3.36±0.02	3.59±0.10	3.32±0.10	2.55±0.01	2.25±0.02
Glyenie	4.50±0.10	4.01±0.01	3.68±0.01	3.40±0.10	4.02±0.10	3.85±0.01	2.63±0.01	2.30±0.10
Alanine	4.02±0.01	3.72±0.01	4.10±0.10	3.87±0.01	4.48±0.01	3.87±0.01	4.35±0.10	4.02±0.01
Cystine	1.52±0.01	1.29±0.01	1.31±0.01	1.38±0.02	1.38±0.01	1.42±0.01	1.45±0.01	1.34±0.02
Valine	5.20±0.10	3.68±0.01	3.92±0.01	4.07±0.01	4.10±0.10	4.20±0.01	4.50±0.01	4.06±0.01
Methronine	1.63±0.02	1.31±0.01	1.31±0.01	1.34±0.01	1.45±0.02	1.48±0.01	1.50±0.01	1.23±0.01
Isoleucine	3.02±0.01	3.13±0.01	3.29±0.01	3.23±0.01	3.23±0.01	3.15±0.01	3.39±0.01	3.01±0.01
Leucine	5.60±0.10	5.81±0.01	6.49±0.01	6.28±0.01	6.69±0.10	6.52±0.01	6.19±0.01	5.48±0.01
Tryosine	2.48±0.02	2.34±0.01	2.65±0.01	2.48±0.01	2.98±0.02	2.63±0.01	3.15±0.01	2.55±0.01
Phenylalanine	4.49±0.02	4.23±0.01	4.31±0.01	4.05±0.01	5.10±0.02	4.87±0.01	4.05±0.02	3.78±0.01
Tryptophan	1.11±0.01	1.09±0.01	1.22±0.01	1.09±0.01	1.32±0.01	1.10±0.01	0.80±0.01	0.50±0.01

There was a decrease in the pH in all the samples (0-72 hours) and increase after 96 hours as shown in Tables 4.14, 4.15, 4.16 and 4.17.

The titrable acidity and volatile acidity decrease throughout the fermentation period in virtually all the samples as shown in Tables 4.18 – 4.25.

Table 4.14 Changes in pH during the 4-Day Fermentation of "ogiri" Samples Produced from Melon Seeds.

Fermentation Period (Hour)	**pH**				
Sample	**1**	**2**	**3**	**4**	**5**
0	6.38±0.29	6.50±0.29	6.90±0.29	6.80±0.29	6.20±0.29
24	6.30±0.36	6.00±0.36	6.80±0.36	6.60±0.36	6.00±0.36
48	5.30±0.64	5.00±0.64	6.50±0.64	6.30±0.64	5.90±0.64
72	5.20±0.57	4.80±0.50	6.20±0.59	6.00±0.59	5.50±0.59
96	6.00±0.46	5.60±0.46	6.80±0.46	6.50±0.46	6.20±0.46

Key: 1, 2, 3, 4 and 5 = different samples of "ogiri" melon seeds.

Table 4.15 pH Changes during 4-Day Fermentation of "ogiri" produced from Castor Oil Seeds.

Fermentation Period (Hour)	**pH**				
	Sample	**8**	**9**	**10**	**11**	**12**
0		7.0±0.27	6.8±0.27	6.6±0.27	6.6±0.27	6.8±0.27
24		6.6±0.13	6.5±0.13	6.4±0.13	6.3±0.13	6.6±0.43
48		5.3±0.54	5.3±0.54	6.1±0.54	5.1±0.54	6.3±0.54
72		5.2±0.45	5.2±0.45	5.0±0.45	5.0±0.45	6.0±0.45
96		6.0±0.39	6.8±0.39	5.9±0.39	6.0±0.39	6.5±0.39

Key: 8, 9, 10, 11 and 12 = different samples of "ogiri" from castor oil seeds.

Table 4.16 pH Changes during 4-Day Fermentation of "ogiri" Samples produced from Fluted Pumpkin Seeds.

Fermentation Period (Hour)	**pH**				
	Sample	**15**	**16**	**17**	**18**	**19**
0		6.0±0.13	6.1±0.13	6.2±0.13	6.0±0.13	6.3±0.13
24		5.9±0.06	6.0±0.06	6.0±0.06	5.9±0.06	6.0±0.06
48		4.9±0.58	4.4±0.58	5.5±0.58	5.8±0.58	5.6±0.58
72		4.8±0.50	4.2±0.50	5.0±0.50	5.6±0.50	4.9±0.50
96		5.7±0.30	5.1±0.30	5.6±0.30	5.9±0.30	5.7±0.30

Key: 15, 16, 17, 18 and 19 = different samples of "ogiri from fluted pumpkin seeds

Table 4.17 pH Changes during the 4-Day Fermentation of "ogiri" Samples produced from *Cucumeropsis* Seeds.

Fermentation Period (Hour)	**pH**				
	Sample	**22**	**23**	**24**	**25**	**26**
0		6.5±0.11	6.6±0.11	6.7±0.11	6.5±0.11	6.4±0.11
24		6.4±0.14	6.4±0.14	6.6±0.14	6.4±0.14	6.2±0.14
48		5.0±0.56	5.3±0.56	6.3±0.56	6.0±0.56	6.1±0.56
72		4.9±0.23	5.2±0.23	5.5±0.23	5.5±0.23	5.6±0.23
96		5.6±0.18	5.5±0.18	5.9±0.18	5.8±0.18	5.9±0.18

Key: 22, 23, 24, 25 and 26 = different samples of "ogiri" from *Cucumeropsis* seeds

Table 4.18 Titrable Acidity during the 4-Day Fermentation of "ogiri" Samples produced from Melon Seeds.

Fermentation Period (Hour)	**Titrable Acidity (g/100ml)**				
Sample	**1**	**2**	**3**	**4**	**5**
0	0.70±0.20	0.40±0.20	0.70±0.20	0.40±0.02	0.42±0.02
24	0.40±0.09	0.30±0.09	0.50±0.09	0.30±0.09	0.30±0.09
48	0.30±0.07	0.21±0.07	0.40±0.07	0.25±0.07	0.25±0.07
72	0.20±0.02	0.20±0.02	0.20±0.02	0.22±0.02	0.25±0.02
96	0.10±0.39	0.10±0.39	0.10±0.39	0.20±0.39	0.14±0.39

Key: 1, 2, 3, 4 and 5 = different samples of "ogiri" from melon seeds.

Table 4.19 Titrable Acidity during the 4-Day Fermentation of "ogiri" Samples produced from Castor Oil Seeds.

Fermentation Period (Hour)	**Titrable Acidity (g/100ml)**				
	Sample **8**	**9**	**10**	**11**	**12**
0	0.41±0.11	0.60±0.11	0.50±0.11	0.70±0.11	0.60±0.11
24	0.30±0.11	0.50±0.11	0.40±0.11	0.60±0.11	0.50±0.11
48	0.28±0.09	0.40±0.09	0.30±0.09	0.50±0.09	0.40±0.09
72	0.20±0.06	0.30±0.06	0.20±0.06	0.30±0.06	0.20±0.06
96	0.10±0.06	0.20±0.06	0.10±0.06	0.20±0.06	0.10±0.06

Key: 8, 9, 10, 11 and 12 = different samples of "ogiri" from castor oil seeds

Table 4.20 Titrable Acidity during 4-Day Fermentation of "ogiri" Samples produced from Fluted Pumpkin Seeds.

Fermentation Period (Hour)	**Titrable Acidity (g/100ml)**				
	Sample	**15**	**16**	**17**	**18**	**19**
0		0.32±0.02	0.36±0.02	0.35±0.02	0.33±0.02	0.36±0.02
24		0.63±0.12	0.37±0.12	0.38±0.12	0.36±0.12	0.37±0.12
48		0.67±0.13	0.38±0.13	0.39±0.13	0.38±0.13	0.38±0.13
72		0.70±0.13	0.40±0.13	0.41±0.13	0.40±0.13	0.39±0.13
96		0.72±0.09	0.53±0.09	0.50±0.09	0.52±0.09	0.50±0.09

Key: 15, 16, 17, 18 and 19 = different samples of "ogiri" from fluted pumpkin seeds.

Table 4.21 Titrable Acidity during the 4-Day Fermentation of "ogiri" Samples produced from *Cucumeropsis* Seeds.

Fermentation Period (Hour)	**Titrable Acidity (g/100ml)**				
	Sample	**22**	**23**	**24**	**25**	**26**
0		0.57±0.09	0.60±0.09	0.38±0.09	0.45±0.09	0.50±0.09
24		0.55±0.09	0.52±0.09	0.35±0.09	0.40±0.09	0.43±0.09
48		0.40±0.08	0.45±0.08	0.25±0.08	0.30±0.08	0.35±0.08
72		0.32±0.05	0.30±0.05	0.20±0.05	0.28±0.05	0.26±0.05
96		0.20±0.02	0.18±0.02	0.15±0.02	0.19±0.02	0.16±0.02

Key: 22, 23, 24, 25 and 26 = different samples of "ogiri" from *Cucumeropsis* seeds.

Table 4.22 Volatile Acidity during the 4-Day Fermentation of "ogiri" Samples produced from Melon Seeds.

Fermentation Period (Hour)	**Volatile Acidity (g/100ml)**				
Sample	**1**	**2**	**3**	**4**	**5**
0	0.05±0.01	0.03±0.01	0.04±0.01	0.05±0.01	0.03±0.01
24	0.07±0.01	0.05±0.01	0.06±0.01	0.06±0.01	0.06±0.01
48	0.09±0.01	0.06±0.01	0.07±0.01	0.07±0.01	0.8±0.01
72	0.12±0.02	0.08±0.02	0.09±0.02	0.09±0.02	0.09±0.02
96	0.13±0.01	0.12±0.01	0.14±0.01	0.12±0.01	0.12±0.01

Key: 1, 2, 3, 4 and 5 = different samples of "ogiri" from melon seeds.

Table 4.23 Volatile Acidity during 4-Day Fermentation of "ogiri" Samples produced from Castor Oil Seeds.

Fermentation Period (Hour)	**Volatile Acidity (g/100ml)**				
	Sample	**8**	**9**	**10**	**11**	**12**
0		0.06±0.02	0.05±0.02	0.07±0.02	0.04±0.02	0.08±0.02
24		0.07±0.01	0.07±0.01	0.08±0.01	0.06±0.01	0.09±0.01
48		0.08±0.02	0.09±0.02	0.12±0.02	0.08±0.02	0.10±0.02
72		0.09±0.02	0.10±0.02	0.13±0.02	0.09±0.02	0.11±0.02
96		0.10±0.02	0.12±0.02	0.14±0.02	0.11±0.02	0.13±0.02

Key: 8, 9, 10, 11 and 12 = different samples of "ogiri" from castor oil seeds.

Table 4.24 Volatile Acidity during 4-Day Fermentation of "ogiri" Samples produced from Fluted Pumpkin Seeds.

Fermentation Period (Hour)	**Volatile Acidity (g/100ml)**				
	Sample	**15**	**16**	**17**	**18**	**19**
0		0.06±0.02	0.02±0.02	0.04±0.02	0.05±0.02	0.03±0.02
24		0.07±0.01	0.05±0.01	0.06±0.01	0.07±0.01	0.05±0.01
48		0.08±0.02	0.06±0.02	0.07±0.02	0.10±0.02	0.07±0.02
72		0.16±0.03	0.13±0.03	0.09±0.03	0.12±0.03	0.09±0.03
96		0.18±0.03	0.14±0.03	0.12±0.03	0.13±0.03	0.11±0.03

Key: 15, 16, 17, 18 and 19 = different samples of "ogiri" from fluted pumpkin seeds

Table 4.25 Volatile Acidity during the 4-Day Fermentation of "ogiri" Samples produced from *Cucumeropsis* Seeds.

Fermentation Period (Hour)	**Volatile Acidity (g/100ml)**				
	Sample	**22**	**23**	**24**	**25**	**26**
0		0.04±0.01	0.05±0.01	0.04±0.01	0.03±0.01	0.05±0.01
24		0.06±0.01	0.06±0.01	0.06±0.01	0.07±0.01	0.07±0.01
48		0.08±0.01	0.07±0.01	0.08±0.01	0.09±0.01	0.08±0.01
72		0.10±0.01	0.08±0.01	0.10±0.01	0.11±0.01	0.10±0.01
96		0.11±0.39	1.00±0.39	0.12±0.39	0.13±0.39	0.12±0.39

Key: 22, 23, 24, 25 and 26 = different samples of "ogiri" from *Cucumeropsis* seeds.

Afflatoxin content was higher in castor oil seed and this is above the limit 20ppb while that of climbing melon has lower afflatoxin content. However, afflatoxin was not detected in the laboratory dehulled seedsand fermented seed as shown in Table 4.26.

Table 4.26 Total Afflatoxin Contents of Laboratory Dehulled, Hawked and Fermented Creeping Melon, Climbing Melon,, Fluted Pumpkin and Castor Oil Seeds.

Sample	**Afflatoxin Concentration (ppb)**		
	Laboratory Dehulled Seeds	**Hawked Seeds**	**Fermented Seeds (**"ogiri"**)**
Creeping Melon Seed	ND	3.0	ND
Climbing Melon Seed	ND	2.0	ND
Castor Oil Seed	ND	> 20	ND
Fluted Pumpkin Seed	ND	1.0	ND

Key: ND = Not Detected

Table 4.27 shows the mineral contents of castor oil seed obtained from various sources. The cadmium content of castor oil seed obtained from Kogi (0.85 ppm), Kebbi (0.90 ppm), Katsina (0.80 ppm), Kano (0.56 ppm), Kaduna (0.58 ppm) and Enugu (0.60 ppm) are higher than their counterpart sources, Abia (0.40 ppm), Anambra (0.35 ppm), Ebonyi 0.50 ppm) and Imo (0.41 ppm). The lead content ranged from 0.01 ppm to 0.40 ppm with that obtained from Katsina and Kaduna registering the highest concentration (0.40 ppm each). The calcium content ranged from 25.00 ppm to 27.00 ppm with that from Kebbi State recording the highest concentration (27.00 ppm). The copper content ranged from 0.26 ppm to 0.44 ppm. The iron content ranged from 0.50 ppm to 0.60 ppm. The magnesium content ranged from 8.0 ppm to 10.20 ppm.

Table 4.28 shows the mineral content of creeping melon seeds obtained from various sources. The cadmium content ranged from 0.28 ppm to 0.98 ppm with that obtained from Kogi State registering the highest concentration (0.98 ppm) while that from Abia State registering the lowest concentration (0.28 ppm). The lead content ranges from (0.01 ppm to 0.18 ppm) with the one obtained from Benue State registering higher concentration (0.18 ppm) while that from Anambra State registering the lowest concentration. The cadmium content ranges from 33.35 ppm to 34.50 ppm with the one from Benue State recoding the highest concentration and that from Kogi recording the lowest concentration. The iron content ranges from 1.29 to 1.52 ppm with the one from Kogi State registering the highest concentration and that from Benue State recording the lowest. The magnesium content ranges from 10.88 ppm to 12.68 ppm with the one from Benue State recording the highest concentration (12.68 ppm) and that from Katsina registering the lowest concentration (10.88 ppm)

Table 4.29 shows the mineral content of fluted pumpkin seeds obtained from various sources. The cadmium content ranges from 0.10 ppm to 0.35 ppm with the one from Kebbi State recording the highest (0.35 ppm) concentration while that from Abia State recording the lowest concentration (0.10 ppm). The lead content ranges from 0.01 ppm to 0.22 ppm with the one obtained from Kebbi State registering the highest concentration (0.22 ppm) while that obtained from Imo State registering the lowest concentration (0.01 ppm). The copper content ranges from 4.01 ppm to 47.89 ppm with the one from Kano registering the highest concentration (47.89 ppm) and that from Kaduna registering the lowest concentration (42.01 ppm). The iron content ranges from 1.12 ppm to 1.80 ppm with the one from Kogi State registering the highest concentration (1.80 ppm) and that from Anambra registering the lowest concentration (1.12 ppm). The magnesium content ranges from 14.05 ppm to 16.05

ppm with the one from Ebonyi State recording the highest concentration (16.05 ppm) and that from Anambra State registering the lowest concentration (14.05 ppm)

Table 4.30 shows the mineral content of climbing melon seeds from various sources. The cadmium content ranges from 0.35 ppm to 1.00 ppm with the one obtained from Kogi State recording the highest concentration (1.00 ppm) and that from Anambra recording the lowest concentration (0.35 ppm). The lead content ranges from 1.85 ppm to 3.60 ppm with the one from Kogi registering the highest concentration (3.60 ppm) and that from Anambra recording the lowest concentration (1.85 ppm). The calcium content ranges from 2.80 ppm to 21.78 ppm with the one from Katsina State registering the highest concentration (21.78 ppm) and that from Kano registering the lowest concentration (20.80 ppm). The copper content ranges from 0.30 ppm to 0.50 ppm with the one obtained from Kano State registering the highest concentration (0.50 ppm) and that from Anambra State registering the lowest concentration (0.30 ppm). The iron content ranges from 0.70 ppm to 1.20 ppm with the one from Kogi State recording the highest concentration (1.2 ppm) and that from Abia and Anambra States recording the lowest concentrations (0.73 ppm each). The concentration of magnesium ranges from 6.81 ppm to 7.50 ppm with the one from Kogi State registering the highest concentration (7.50 ppm) and that from Anambra recording the lowest concentration (6.81 ppm).

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Table 4.27 Mineral Content of Castor Oil Seed Samples from various Sources

Sample Source	**Mineral Content (ppm)**					
(State)	**Calcium**	**Copper**	**Cadmium**	**Lead**	**Iron**	**Magnesium**
Abia	26.00	0.30	0.40	0.01	0.48	9.25
Anambra	25.50	0.29	0.35	0.02	0.49	9.30
Benue	25.00	0.31	0.65	0.25	0.47	9.26
Ebonyi	26.50	0.28	0.50	0.20	0.54	8.95
Enugu	26.50	0.35	0.60	0.05	0.46	8.98
Imo	25.20	0.26	0.41	0.02	0.48	8.86
Kaduna	26.60	0.42	0.58	0.40	0.50	8.80
Kano	26.80	0.33	0.56	0.22	0.52	9.60
Katsina	25.80	0.44	0.80	0.40	0.45	9.10
Kebbi	27.00	0.32	0.90	0.30	0.60	10.00
Kogi	25.00	0.33	0.85	0.33	0.51	10.20

Table 4.28 Mineral Contents of Creeping Melon Seed Samples from various Sources.

Sample Source	**Mineral Content (ppm)**					
(State)	**Calcium**	**Copper**	**Cadmium**	**Lead**	**Iron**	**Magnesium**
Abia	34.20	0.95	0.28	0.01	1.36	11.65
Anambra	34.00	0.93	0.30	0.02	1.34	11.66
Benue	34.50	0.90	0.70	0.18	1.29	12.68
Ebonyi	33.90	0.89	0.60	0.12	1.31	11.59
Enugu	35.00	0.97	0.70	0.10	1.37	11.62
Imo	33.80	0.95	0.50	0.05	1.33	11.64
Kaduna	34.10	0.98	0.67	0.10	1.36	11.70
Kano	33.95	1.20	0.80	0.12	1.32	12.00
Katsina	35.05	0.99	0.95	0.13	1.30	10.88
Kebbi	33.50	1.02	0.85	0.14	1.35	10.90
Kogi	33.35	1.00	0.98	0.13	1.52	11.94

Table 4.29 Mineral Contents of Fluted Pumpkin Seed Samples from various Sources

Sample Source	**Mineral Content (ppm)**					
(State)	**Calcium**	**Copper**	**Cadmium**	**Lead**	**Iron**	**Magnesium**
Abia	46.70	0.15	0.10	0.03	1.16	14.15
Anambra	42.20	0.10	0.11	0.02	1.12	14.05
Benue	47.80	0.23	0.29	0.09	1.35	14.95
Ebonyi	43.00	0.20	0.24	0.07	1.62	16.05
Enugu	42.90	0.25	0.23	0.09	1.35	15.30
Imo	46.99	0.18	0.10	0.01	1.60	15.60
Kaduna	42.01	0.24	0.31	0.15	1.40	16.00
Kano	47.89	0.30	0.20	0.10	1.50	15.20
Katsina	43.03	0.28	0.25	0.12	1.30	15.00
Kebbi	44.49	0.35	0.35	0.22	1.42	16.02
Kogi	44.30	0.25	0.30	0.20	1.80	15.98

Table 4.30 Mineral Content of Climbing Melon Seed Samples from various Sources.

Sample Source	**Mineral Content (ppm)**					
(State)	**Calcium**	**Copper**	**Cadmium**	**Lead**	**Iron**	**Magnesium**
Abia	21.30	0.35	0.38	1.98	0.73	6.81
Anambra	21.20	0.30	0.35	1.85	0.73	6.85
Benue	21.26	0.38	0.74	3.43	0.79	7.15
Ebonyi	21.50	0.40	0.38	2.00	0.82	6.88
Enugu	22.00	0.37	0.50	2.75	0.98	7.50
Imo	20.95	0.41	0.40	2.85	0.75	7.12
Kaduna	21.10	0.36	0.65	2.99	0.85	7.08
Kano	20.80	0.50	0.70	2.80	0.89	6.95
Katsina	21.78	0.31	0.59	3.05	1.05	7.00
Kebbi	21.21	0.40	0.80	3.50	1.00	7.20
Kogi	22.23	0.40	1.00	3.60	1.2	7.50

Table 4.31 Shows the mean bacterial and fungal counts (cfu/g/ml) of samples from the production stages of "ogiri" from creeping melon, climbing melon, fluted pumpkin and castor oil seeds.

Production Stage	**Heterotrophic Bacterial Count ($x10^6$)**	**Coliform count ($x10^6$)**	***Salmonella-Shigella* Count ($x10^6$)**	**Fungal Counts ($x10^4$)**
Raw seed	5.0	4.2	3.5	4.2
Boiled seed	4.8	3.4	3.0	3.7
Fermented seed	4.9	4.3	3.4	4.3
Nasal swab	5.5	3.4	NG	5.2
Skin swab	3.5	3.2	NG	3.0
Mortar	4.5	2.4	6.5	1.4
Pestle	4.4	2.2	5.8	1.5
Water	1.2	2.3	2.1	2.0
Leaves	2.5	2.5	4.4	1.2
Strings	3.8	1.2	1.0	2.0

FAO (1979) Maximum acceptable limit is 1.00×10^5 cfu/g/ml

Table 4.32 Shows the various bacterial and fungal isolates detected from the samples at various stages of production of "ogiri" from creeping melon, climbing melon, fluted pumpkin and castor oil seeds.

Production Stage	**Bacteria Isolated**	**Fungi Isolated**
Raw seeds	*E. coli*, *Staph. aureus*, *Bacillus fusiformis*, *Pseudomonas* spp and *Proteus* spp	*Aspergillus fumigatus*, *Penicillium* spp, *Mucor* spp, *Trichoderma reesei*, and *Aspergillus terreus*
Boiled seeds	*Staphylococcus aureus* and *Streptococcus* spp	*Mucor* spp
Fermented seeds	*E. coli*, *Staph. aureus*, *Flavobacterium* spp, *Micrococcus* spp, *Bacillusfusiformis, Shigella* spp, and *Lactobacillus*	*Mucor* spp
Nasal swab	*Staph. aureus, Streptococcus* spp, *Bacillus* spp and *Pseudomonas* spp	*Penicillium* spp, *Aspergillus terreus*
Skin swab	*Staph. aureu*s and *E. coli*	*Mucor* spp
Mortar	*Salmonella* spp, *Staphaureus*,	*Mucor* spp, *Penicillium* spp, *Aspergillus fumigatus*
Pestle	*Salmonella* spp, *Shigella* spp, *Staph. aureus*	*Mucor* spp, *Penicillium* spp
Water	*Pseudomonas, E. coli*, *Proteus spp*, *Enterobactercloacae, Leclercia adecarboxylata, Vibrio*	*Aspergillus* spp, *Penicillium* spp
Leaves	*Actinomyces* spp, *Klebsiella pneumoniae, E. coli*	*Trichoderma ressei*, *Mucor* spp, *Aspergillus fumigatus*

Table 4.33 Mean heavy metal contents (ppm) of the samples at various stages of "ogiri" production from creeping melon, climbing melon, fluted pumpkin and castor oil.

Production Stage	**Copper**	**Lead**	**Cadmium**
Raw material	0.48	0.82	0.53
Sorted seeds	0.40	0.75	0.45
Boiled seeds	0.33	0.35	0.30
Water		NP	NP
Fermented seeds	0.33	0.35	0.30

Key: NP = Not Present

NAFDAC acceptable limits for Heavy Metals in food condiments (copper = 5.0 ppm, Lead = 0.20 ppm and Cadmium = 5.0 ppm)

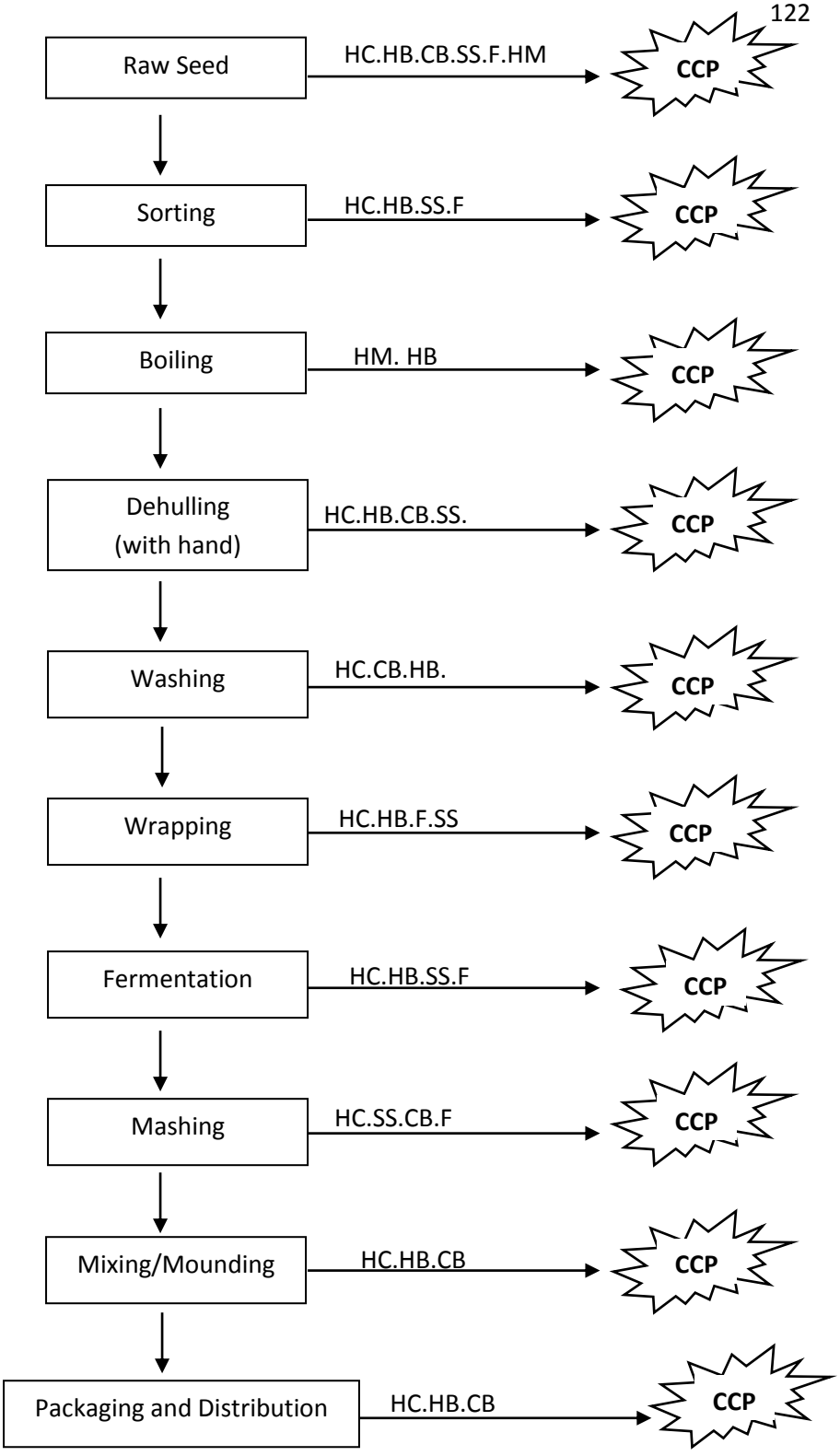

Figure 4.1 Critical control points from the production stages of "ogiri" from creeping melon, fluted pumpkin, castor oil and climbing melon seeds.

Key: CCP = Critical Control Point; HC = High Count; HB = Heterotrophic Bacteria; CB = Coliform Bacteria; SS = Salmonella Shigella; F = Fungi; HM = Heavy Metal

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

There is higher microbial counts in the "ogiri" obtained from the local producers than in the laboratory prepared "ogiri" though similar microbial flora was observed in both (Tables 4.1 to 4.4). The high microbial counts in the "ogiri" obtained from local producers could be as a result of poor hygienic practices and poor sanitary quality of processing utensils, water and packaging materials. A similar observation was made by (Odibo and Umeh, 1988; Odibo *et al.*, 2012 and Dimejesi and Iheukwumere, 2014).

A great number of bacterial genera identified as *Pseudomonas plecoglossida, Bacillus fusiformis, Klebsiella pneumoniae, Enterobacter cloacae, Lactobacillus* spp, *Leclercia adecarboxylataStreptococcus* spp, *Proteus* spp, *Escherichia coli, Salmonella* spp,*Shigella* spp, *Vibrio, Flavobacterium, Micrococcus* spp, *Pediococcus* spp *and Actinomyces* spp were discovered. Fungi isolated were *Aspergillus fumigatus, Trichoderma reesei, Aspergillus terreus, Penicillium* spp and *Mucor* spp.

The leaves used in wrapping the fermenting seeds and the mortar and pestle used in mashing the seeds constitute the major sources of bacteria particularly *Actinomyce*s spp and *Salmonella* spp from the leaves of mortar, respectively. The isolation of *Bacillus* spp, *Pseudomonas* spp, *Klebsiella* spp, *Staphylococcus aureus, Pediococcus* spp and *Lactobacillus* spp (Table 4.32) is in line with the findings of Odibo *et al.*(2012) and earlier reports of Odunfa(1981a and b)

The population of pathogenic organisms may be attributable to intrinsic factors of "Ogiri" samples such as availability of nutrients, pH, water activity (a_w), lack of competing organisms and extrinsic factors which include the storage temperature. This observation is still in line with that of David and Adenibigbe (2010).

The disappearance of fungi after three days fermentation of "ogiri" samples (Table 4.10) shows that fungi are not favoured by the near alkaline pH of the process (alkaline fermentation) and anoxic fermentation. This agrees with the observation made by Dimejesi and Iheukwumere(2014). The progressive increase in the microbial load was also observed by David and Aderibigbe(2010).

In traditional fermented foods preparation, microbes are used to prepare and preserve food products (Achi, 2005) and hence fermentation of food has many advantages such as

improvement of nutritional value and protection against bacterial pathogens (Gadaga *et al.*, 2014).

The isolation of coagulase positive *staphylococcus aureus* from fermenting seeds is of public health concern as the organism is known to cause food poison (Frazier and Weshoff, 2000). This organism was isolated from nasal and skin swabs of the producers and hence was observed to contaminate the products, "ogiri". The presence of *Klebsiella*, a coliform could constitute a health risk since some species of this genus are associated with the disease of man. This observation was also made by (Odibo *et al.*, 2012) who also expressed the expectation that high heat treatment subjected to "ogiri" and "ogiri okpei" respectively during cooking will destroy these microorganisms and possibly any toxin in the condiment. Therefore there is the risk of using "ogiri" in the preparation of cold ready-to-eat foods such as abacha.

The presence of *E. coli*, an indicator organism was observed to come from the water used in the reconstitution of "ogiri". *Enterobacter aerogenes* and *Enterobacter cloacae* are widely distributed in water sewage, soil and on vegetables and are occasionally found in faeces and respiratory tract of man (Arora, 2009). These organisms have been reported to occasionally cause meningitis and septicaemia. Therefore, the isolation of *Enterobacter cloacae* from "ogiri" samples may be attributed to the water used in reconstitution, the unwashed leaves usually used in wrapping "ogiri" and poor hygienic practices by the handlers. *Pseudomonas* spp are also widely distributed in soil, water and sewage, so its presence in "ogiri" is understandable.

Actinomyces, a filamentous anaerob to micro aerophilic, Gram positive, non-spore forming rods is typically found in the soil, decaying organic matter and can also be found in oral cavities, dental plagues and intestinal tract of mammals (Goodfellow and William, 1983). The isolation of *Actinomyces* is observed to come from the leaves used in wrapping "ogiri" since the leaves are usually collected from the bush and used for wrapping unwashed.

Salmonella spp, isolated were typically from the mortar and pestle used in mashing. It was also observed that the local producers of "ogiri" use these utensils several times without washing and the cracks in these utensils serve as a hiding/breeding area for the organisms and probably other contaminants. Most of isolates could not be identified to species levels using morphological and biochemical characteristics. So, DNA sequence based identification was used to identify species within the complex.

The isolation of some pathogens from "ogiri" portends a great danger to the consumption of this product. However, Odibo *et al.* (2012) also reported that high heat

treatment subjected to “ogiri” and “ogiri okpei” during cooking will destroy these microorganisms and possibly the toxins elaborated in the condiment.

The increase in pH during fermentation has also been reported by (Aderibigbe and Adebayo, 2012). The increase in moisture content of the processed (fermented) products agree closely with the report of (Omafuvbe *et al.*, 2004) and this may be as a result of the decomposition of the fermenting bacteria on the products.

The increase in pH during the fermentation of melon seeds, climbing melon seeds, castor oil seeds and fluted pumpkin seeds which had been reported to aciduric could have contributed to the poor growth of *Lactobacillus* spp (Aderibigbe and Adebayo, 2002). The increase in pH could also be attributed to the ability of the fermenting organisms to degrade protein (Onawola *et al.*, 2011); Berber and Achiwehu, (1992) also reported that the amino acids produced due to protein metabolism are responsible for gradual pH increase leveling off towards 7.5-8.0 and the pH increase may also be due to formation of ammonia from amino acids.

The decrease in the amount of protein with increase in the population of organisms suggest utilization by the organisms and this agrees with the findings of (Enujiugha, 2003 and Olaofe *et al.*, 1993) and hence attributed to the high proteolytic activities of the fermenting organisms generating free amino acids (Onawola, 2011). However, boiling as a processing technique reduce seeds’ crude protein while the combination of boiling and fermentation increases protein content (Igwe *et al.*, 2012).

The decrease in crude fibre and ash content are in agreement with that of (David *et al.*, 2010) for “ogiri” produced from different melon seeds.

The increase in the volatile acidity in virtually all the samples (Table 4.11) indicates similar increase in the production of free fatty acid by the lipolytic fermenting organisms and this agrees with the findings of Dimejesi and Iheukwumere (2014).

The detection of total aflatoxin in the hawked seeds is undesirable as this may be caused by poor house-keeping and storage conditions. None was detected in the fermented products as the causative organisms are destroyed during fermentation. The concentration of volatile acid serve as an indicator of food spoilage and shows that the food has been infected with microorganisms (Bielig *et al.*, 1976).

Food crops when grown in the soil contaminated with lead absorb the element (lead) which adversely affect the consumers of the products.

The high level of lead may be attributed to the raw materials source because most of the substrates used in producing “ogiri” are produced in the affected areas and are distributed

all over the country. Lead and Copper are used for making pesticides which is used for crop preservation and pest control. These elements penetrate into food stuff which when consumed cause cancer. One of the sources of cadmium is build up of cadmium levels in water, air and soil from industrial areas. Crops grown in cadmium contaminated irrigation water is likely to be contaminated with cadmium.

Acute exposure to cadmium may cause flu-like symptoms like chills, fever, and muscle ache sometimes referred to as "Cadmium blues". High levels may cause trachea-bronchitis and pulmonary edema and can also lead to kidney problems which can be fatal.

Cadmium exposure has also been reported to cause kidney stone and kidney damage inflicted by cadmium poisoning is irreversible. However, a decrease in the cadmium, copper, lead, calcium and magnesium contents was observed in processed product, "ogiri". A similar observation was also made by Aduanya *et al.* (2013) in processing African breadfruit (*Treculia africana*) seeds.

Heat has little or no effect on mineral content of food hence, they are said to be heat stable. The slight decrease in the mineral content (Table 4.12) could be as a result of leaching out minerals when food is cooked in boiling water.

The presence of lead and cadmium poses a public health hazard to the consumers. The possible source of lead and cadmium could be from the water around mining areas due to industrial effluent used in reconstitution, soil contaminated with these elements, poor mining and hygienic practices (Dada and Akpan, 2015). The concentration of lead in unprocessed samples are higher than that in processed (fermented) samples with that of Cucumeropsis (2.80ppm) above the NAFDAC limit (0.2 ppm) for spices. The concentration of Cadmium in both unprocessed and processed samples (18.78 – 72.70 ppm) is above the NAFDAC limit (5.00 ppm) for spices. However, the general decrease in the mineral contents was also observed by (Aduanya *et al.*, 2013) in boiling of African breadfruit (*Treculia africana* seeds). The increase in iron content in the processed samples is in line with that of (Aduanya *et al.*, 2013) who pointed that roasting of breadfruit increased the mineral contents of the samples especially iron and copper. Boiling has been reported to decrease iron and copper contents compared with the raw samples (Ayoola and Adeyeye, 2010).

The significant decrease in the copper content from processed and unprocessed (< 0.05) samples is in line with the findings of (Ayoola and Adeyeye, 2010). Mineral analysis are essential to guarantee the quality of any food product. Some minerals are vital for the proper functioning of the body while others are toxic (Abdabasit *et al.*, 2012). The higher cadmium content in all the unprocessed samples than in the processed (boiled) samples

suggests that the cadmium content can further be reduced by large period of boiling. In the same vein, the concentration of lead were higher in the raw (unprocessed) samples than in the processed (boiled) samples. The higher heavy metal contents especially cadmium and lead in castor oil, creeping melon, fluted pumpkin and climbing melon obtained from Kogi, Kebbi, Katsina, Benue, Kano and Kaduna States of Nigeria may be attributed to environmental pollution due to industrial effluent.

Glutamic acid and aspartic acid are the most concentrated amino acids (Table 4.13) and this observation is in agreement with the earlier report trend of glutamic acid aspartic acid being the most concentrated amino acids in oil seeds (Igwe *et al.*, 2012, and Ugwu *et al.*, 2012). Methionine was the least concentrated in all the samples and the low concentration of methionine is in consonance with earlier reported least concentration of methionine in some raw and processed legumes (Khattab *et al.*, 2009 and Ojiakor *et al.*, 2012).

The result of moisture analysis shows that the moisture content is higher in the processed samples of "ogiri" than in the raw seeds (substrates). It is believed that materials containing high moisture content has less storage stability than those with lower moisture content. Adegunwa *et al.* (2011) while carrying out research on effect of processing on the nutritional contents of yam and cocoyam tubers observed that the processed samples with moisture content higher than 12% has less storage stability than those with lower moisture content.

The higher moisture content in the processed product could be caused by the quantity of water used in the reconstitution and the variation could also be as a result of variation in the dehulling method. The increase in moisture contents of the processed fermented products agrees closely with the report of David *et al.* (2010) and Adegunnwa *et al.* (2011) on microbiology and proximate composition of "ogiri" produced from different melon seeds. Though the moisture content observed in this work is higher with that observed by David *et al.* (2010) and this may be due to differences in the process method and also the methodology used in carrying out the analysis as well as the efficiency of the equipment used in the analysis.

There was a general decrease in the crude fibre content in all the samples of castor oil seed, *Cucumeropsis manii*, fluted pumpkin seed and melon seed. David *et al.* (2010) also observed similar decrease in the crude fibre content but the crude fibre contents are higher in virtually all the samples (*Cucumeropsis manii, Citrillus lenctus* and *Citrillus vulgaris*). However, in this work, *Cucumeropsis manii* recorded the lowest fibre content in both

processed and unprocessed samples and this could be attributed to the state of seeds before analysis and also the method and equipment used in the analysis.

There is significant increase in the free fatty acid content in all the samples. Fat is found in most food groups and foods containing fat generally provide a range of different fatty acids both saturated and unsaturated.

The balance of Omega 3 and Omega 6 polyunsaturated fatty acids in western diets has changed substantially over last 100 years as the two families of polyunsaturated fatty acids share a common metabolic pathways. Concern has been raised that this might be detrimental to health. It is advisable to increase our consumption of long chain Omega 3 polyunsaturated fatty acids and decrease the intake of saturated fatty acids which increases the level of cholesterol. High free fatty acid bring about oxidative hydrolytic rancidity in food which results in off flavour and colour and these are not desirable in food. Also this chemical process (decomposition of fat by hydrolysis or oxidation) destroys nutrients in food especially vitamins. However, Odibo *et al.* (2008) made similar observation that a disadvantage of high concentration of unsaturated fatty acids in the product would be susceptible to rancidity, since the fats are less stable than saturated fats and this should be taken into consideration in designing procedures for preservation of food.

Vegetable oils undergo changes in terms of chemical and physical properties when they interact with food or atmosphere. Some food processing techniques can affect fatty acid composition of oils when hardly subjected to successive heating (Lee *et al.*, 2004). Types of reaction that are known to lead to degradation of vegetable oils include polymerization, oxidation and hydrolysis. The fatty acid composition of oils can be indicator of its stability, physical properties and nutritional values.

The ash contents were low in virtually all the samples and this is in agreement with the findings of David *et al.* (2010) on the "microbiology and proximate composition of 'ogiri'". The high carbohydrate content observed in his work contrasted his findings on the carbohydrate content. However, the ash observed in this work is higher than the NAFDAC limit for ash content 0.3 – 1.2% for spices.

The appreciable concentration of Lysine (3.79g/100g), Histidine (3.0g.100g), Valine (5.20g/100g), Isoleucine (3.02g/100g), Leucine (5.60g/100g) and Phenylalanine (4.49g/100g) in both processed and unprocessed samples is very important from the nutrition point of view as these are among the essential amino acids. Similar observation was also made by Odibo *et al.* (2008).

The concentrations of methionine, cystine and tryptophan were low 1.63g/100g, 1.52g/100g and 1.11g/100g, respectively and this disagrees with observation made by Odibo *et al.* (2008).

Glutamic acid was recorded as the highest concentrated amino acids in both unprocessed and fermented samples. The sodium salt of L-glutamic forms the active flavouring agent in most commercial seasoning agents (e.g. Maggi cubes or sauce). This observation is in line with that made by Odibo *et al.* (2008).

Abdalbasit *et al.* (2012) reported increase in the percentage of sulphur containing amino acids after boiling safflower seed but the reduction in the concentration of the sulphur containing amino acids (cystine and methionine) contrasted this observation. This is probably due to active metabolism of the compounds by the fermenting organisms and this was earlier reported by (Odibo *et al.*, 2008).

There is a general decrease in the amino acid content of the processed samples ("Ogiri") except in the processed (fermented) castor oil seeds where increase in the amino acids (Arginine, Isoleucine and Leucine) was observed. Similarly, there was increase in lysine, proline, valine, and methionine contents of processed melon seed "ogiri".

The general reduction in the concentration of most amino acids was also observed by Odibo *et al.* (2008) in the fermentation of *Prosopis africana* seeds for ogiri okpei production. This reduction in amino acid contents after processing (fermentation) suggests active metabolism of these components by the fermenting bacteria and this is also in consonance with the findings of Allagheny *et al.* (1996).

The most abundant amino acid and highly concentrated amino acids in the unprocessed (unfermented) seeds were glutamine (glutamic acid), aspartic acid and arginine.
Ojinnaka and Ojimelukwe (2012) reported that free amino acids such as glutamic acid and aspartic acid may have contributed to the formation of flavour during the fermentation of *Ricinus communis*. It is well known that glutamic acid and aspartic acid contribute to the pleasant umami taste or savoury enhancement in food.
Lioe *et al*. (2004, 2007) carried out chemical and sensory studies on savoury fractions obtained from soy sauces and phenylalanine as well as NaCl and glutamic acid in the fractions. A potential synergistic effect of umami among free glutamic acid, salt and phenylalanine (bitter amino acid) were observed.

Proteolysis has been reported as the main metabolic activity during the fermentation of African locust bean which also contributes to the development of texture flavour of the fermented products (Folasade *et al.*, 2008).

Kpikpi *et al*. (2009) also reported that amino acids play a major role in the taste and flavour development in food.

5.2 **Conclusion**

The organisms associated with the fermentation of "ogiri" from various substrates have been established. The amino acid profile of fermented "ogiri" from different substrates has also been elucidated from the study.

Heavy metals such Cd^{2+}, Pb^{12+}, Cu^{2}, Fe^{2+} and Mg^{2+} were detected on unprocessed and processed substances for "ogiri" production. In addition aflatoxin was found majorly in the locally processed "ogiri" samples. The hazard analysis revealed sorting, washing, fermentation, mixing, boiling, packaging and raw material as critical control points.

Apart from these controls specified as critical control points for "ogiri", the quality of the water used in reconstitution, the cleanliness and sterilization procedures and personal traffic hygiene were also analyzed within the HACCP system to improve the effectiveness of system.

5.3 **Recommendations**

Since women who are largely involved in the production of "ogiri" are ignorant of good house-keeping, good hygiene practices and good manufacturing practices, which are prerequisite operation needed for the successful implementation of HACCP, it becomes a challenge. It is therefore recommended that all the regulatory agencies relating to food safety such as NAFDAC, SON, National Codex Committee, Ministry of Agriculture, states and local governments should join hands in order to:

1. Protect the public against injury to health through the consumption of unwholesome food.
2. Educate the populace on sound hygienic and safety practices.
3. Conduct public health surveillance of food premises, food handlers and equipment used for food processing.
4. Restrain the sale of foods which are not hygienically prepared, adulterated and contaminated.

   Finally, people should boil "ogiri" before using to prepare ready-to-eat foods such as abacha.

5.4 Contribution to Knowledge

This study has shown that:

1. "Ogiri" samples especially the locally processed samples contain some pathogenic bacteria.
2. Aflatoxin can be detected in "ogiri".
3. Heavy metals can be found in unprocessed and processed substrate for "ogiri" production.
4. The critical points for "ogiri" production are dehulling, raw materials, mixing, packaging, fermentation and washing.
5. The amino acid profile of the unfermented and fermented "ogiri" samples were elucidated.

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