MICROBIOLOGICAL QUALITY ASSESSMENT OF FORMULATED INFANT FLOUR MEALS AVAILABLE IN LOCAL SHOPS IN MOROGORO MUNICIPALITY

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PUBLIC HEALTH AND FOOD SAFETY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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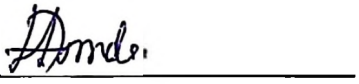
ABSTRACT

This study was conducted to assess the microbiological quality of formulated infant flour meals sold in local shops in Morogoro Municipality. A total of 105 infant flour bags from 16 different shops were collected and assessed. All the infant flour meals collected in this cross-sectional study were cereal based for which case the raw materials were obtained locally. The main raw material used as observed on the labels of the packages were rice, maize, soybean, groundnuts, finger millet, sorghum, wheat, carrot, vegetable and fish. The samples were analysed for total plate count (TPC), for *Escherichia coli* and fungal (moulds) contaminants using enumeration methods. Results of all sampled infant flour meals were in the range of (3.43 ± 0.71) $x10^4$cfu/g to (4.09 ± 1.77) $x10^5$ cfu/g for total plate count, 0 to 71.22 ± 58.13 MPN/g for *Escherichia coli* and (2.32 ± 0.60) $x10^3$cfu/g to (8.01 ± 1.10) $x10^4$cfu/g for moulds. Microscopic observation for moulds indicated the presence of *Aspergillus* spp., *Cladosporium* spp. and *Penicillium* spp. The findings of this study indicate a huge load of microorganisms as observed in the total plate count which is above the required range of the Tanzanian standard for processed cereal based weaning foods (TZS 180, 1983) for all flour meals. After statistical analysis using Open Epi (ANOVA) program, it was revealed that there is a significant difference ($p<0.05$) in microbiological quality between brands of infant flour meals. The findings from this study pose an alarm to the responsible authorities dealing with food quality issues bearing in mind that infants are at high risk of getting health problems associated with consumption of the infant meals. There is thus a need for responsible government institutions to strengthen efforts of safe food production from farm to final stage of consumption and initiate sustainable intervention measures to improve the microbiological qualities of formulated infant flour meals that will comply with set standards.

DECLARATION

I, ABEL MWAKASONDA, do hereby declare to the Senate of Sokoine University of Agriculture that this is my original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.



29/04/2014

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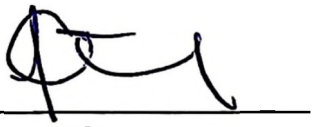

1/5/2014

Prof. MTAMBO, M.M.A. — Date

(Supervisor)

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DEDICATION

This work is humbly dedicated to my beloved wife, Jesca Laurent and my son Patrick Abel Clement Mwakasonda, for their love and encouragement throughout the time of my studies.

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LIST OF ABBREVIATIONS AND ACRONYMS

A_{1-15}	Sample A identification number marked One to Fifteen
ANOVA	Analysis of Variance
ATCC	American Type Culture Collection Centre
B_{1-15}	Sample B identification number marked One to Fifteen
BPW	Buffered Peptone Water
C_{1-15}	Sample C identification number marked One to Fifteen
CAC	Codex Alimentarius Commission
CFU	Colony Forming Units
D_{1-15}	Sample D identification number marked One to Fifteen
DS	Double Strength
E_{1-15}	Sample E identification number marked One to Fifteen
EC	*Escherichia coli*
F_{1-15}	Sample F identification number marked One to Fifteen
FAO	Food and Agriculture Organization of the United Nations
F_C	F- statistic calculated
FIFM	Formulated Infant Flour Meal
G_{1-15}	Sample G identification number marked One to Fifteen
H_{01}	First null hypothesis
H_{02}	Second null hypothesis
H_{A1}	First alternative hypothesis
H_{A2}	Second alternative hypothesis
ID	Identification
Log_{10}	Logarithm to the base of ten
LPCB	Lacto phenol Cotton Blue

LST	Lauryl Sulphate Tryptose
MPN	Most Probable Number
NBS	National Bureau of Statistics
NI	Not Indicated
PCA	Plate Count Agar
SD	Standard deviation
SPP	Species
SS	Single Strength
SUA	Sokoine University of Agriculture
TBS	Tanzania Bureau of Standards
TFDA	Tanzania Food and Drugs Authority
TFNC	Tanzania Food Nutrition Centre
TMA	Tanzania Meteorological Agency
TPC	Total Plate Count
TW	Tryptone Water
TZS	Tanzanian Standard
UNICEF	United Nations Children's Fund
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Formulated infant flour meal (FIFM) is one among the food categories based on cereals which contains essential nutrients such as carbohydrates, fats, proteins, vitamins and minerals and are usually sourced from plant or animal products (Amankwah *et al.*, 2009). Cereals, root and tuber crops are the staple food of people in the tropics, and provide about 75% of their total caloric intake and 67% of their total protein intake (Fasasi *et al.*, 2005). Cereal based foods are a major source of inexpensive dietary energy and nutrients in developing countries (Opere *et al.*, 2012). Among the cereals most consumed in Tanzania in various formulations are millets, maize, wheat, rice, and sorghum as well as root crops like cassava and potatoes (Mosha *et al.*, 2000; Mamiro *et al.*, 2005). Legume family crops mostly used in production of formulated infant flour are soybeans and groundnuts (Aworh, 2008).

Flours produced from cereals, legumes or tubers have a nutritional value inferior to those produced from a combination of cereals, legumes or tubers (Laura *et al.*, 2011). The raw materials used to prepare formulated infant flour meals usually pass through different processes during preparation into food (Forsythe and Hayes, 1998). The aim is to produce food of high quality in order to avoid health risks that may be associated with the final product. Microbiological contamination is one of the problems that have to be avoided because of the health problems that may cause to the consumer (Forsythe and Hayes, 1998). Consumption of food e.g. Formulated infant flour that was originally contaminated with mycotoxins produced by some mould species may result in various health problems

one of them being neurological problems causing death to a consumer. (Madigan and Martinko, 2006).

Formulated infant flour meals produced by sophisticated technologies are costly and are not within the reach of most entrepreneurs in rural and urban cities of developing countries (Badau *et al.*, 2005). Most families have to depend on the local material and technology for the preparation of weaning food and the alternative cheap technology for preparation of low cost weaning food is malting and the finished product is seldom free of micro-organisms (Mosha and Svanberg, 1983). Therefore, there is a need to evaluate the weaning food produced to ascertain its microbiological quality (Badau *et al.*, 2005).

Maternal and child nutrition is one key factor that determines the outcome of proper human development and significantly has an influence on the quality of life in later years (Ayieko and Anyango, 2011). In the developing world where a big number of people are low income earners, the time of weaning is considered a critical period in a life of children (Afifi *et al.*, 1998). In various parts of developing countries like Nigeria, children weaned between the ages of 3-24 months, are mostly fed on locally available staples without considering properly the nutritional requirements of the infant (Akinrele and Edwards, 1971; Ketiku and Smith, 1984; Achienewhu, 1987 and Adeyemi *et al.*, 1989). These are mostly contributed by several factors some of them being poor nutritional education, poor household income and high cost of commercial weaning foods that in most cases are manufactured using high technology and sometimes are sold in sophisticated packages (Okafor *et al.*, 2008).

It has also been observed in other parts of West African countries that, exclusive breastfeeding is usually adequate up to three to four months of age (Ayo *et al.*, 2011).

After this period most infants are supplemented with special formulated infant flour meal to support their growth (Ayo *et al.*, 2011). Many studies have reported that the incidence of diarrhoeal disease is high during weaning period (Rowland and McCollum, 1977; Mata, 1978 and Barrel and Rowland, 1979) whereby enteropathogens have been detected in weaning foods in different parts of the world the most common being *E. coli* (Henry *et al.*, 1990).

1.1.1 Available information in Tanzania

Tanzania is one of the developing countries located in the Sub-Saharan eastern part of the African continent. The country is ranked as 10th in its contribution to the World's chronically undernourished children whereby 44% of children under 5 years old suffer from stunted growth which indicates chronic undernourishment, about 4% children are wasted being a sign of acute undernourishment, 22% children are underweight and 72% are anemic (UNICEF, 2009a). Exclusive breastfeeding for the first 4 to 6 months protects the infant from nutritional deficiencies and decreases the stress of infection (Mamiro *et al.*, 2004).Low adherence to exclusive breastfeeding and very early or delayed introduction of complementary foods is a common occurrence among children in Tanzania (Muhimbula and Issa-Zacharia, 2010). Complementary feeding generally starts early with 7% of children below 2 months, 32% of children between 2 to 3 months and 58% of children between 4 to 5 months in Tanzania and an estimated 41% of infants below 6 months of age are exclusively breastfed (NBS and ORC Macro, 2005). Majority of the infants in developing countries are introduced to cereal-based complementary foods before the recommended 6 months age (Onyango, 2003). Six months age is recommended for infants to be supplemented with 'safe and nutritionally adequate' complementary foods (Onyango, 2003). In Morogoro municipality, mothers from the medium income groups introduced complementary foods to children at an early age of 1 to 2 months as compared

to high income group who introduced such foods at an age of 5 to 6 months (Shirima *et al.*, 2001). Low nutrient density in weaning foods has also been a major cause of undernourishment among infants and young children in developing countries (Mosha and Vicent, 2005).

Infants and young children are very susceptible to food borne-diseases and if they consume contaminated foods, they are likely to contract infections or intoxications leading to illness and sometimes death (Muhimbula and Issa-Zacharia, 2010). Numerous studies in developing countries have shown that weaning foods prepared under unhygienic conditions are easily contaminated with pathogenic agents (Muhimbula and Issa-Zacharia, 2010). The pathogens are major risk factor forcausing diseases such as diarrheal (Muhimbula and Issa-Zacharia, 2010).The pathogens isolated include bacteria such as *Escherichia coli*, *Shigella*spp., *Salmonella* spp., *Vibrio cholera*, *Campylobacter jejuni* (Black *et al.*, 1989; Gomes, 1991), *Bacillus cereus*, *Staphylococcus aureus* and *Clostridium perfringens*. Infections caused by pathogenic *E. coli* strains are the commonest illnesses and are responsible for up to 25% of all diarrhoeal episodes in developing countries (Motarjem *et al.,* 1993).Studies conducted in different areas in Tanzania have highlighted some factors associated with inappropriate complementary feeding practices to infants who are introduced to complementary foods at inappropriate age, infrequent feeding, poor nutritional quality of the complementary foods and furthermore, most foods are unsafe hygienically (Mosha *et al.*,2000; Mamiro *et al.*, 2005 and Nyaruhucha *et al.*, 2006). There is not enough information on the microbiological quality of formulated infants flour meals sold in local shops in Morogoro municipality and therefore the purpose of this study was to assess the microbiological quality of the products.

1.2 Problem Statement and Justification of the Study

Various formulations of nutritious flour meals are produced to target certain groups of individuals including growing children, sick people and those with nutritional problems, nevertheless, little is known on the microbiological quality of the formulations (Amankwah *et al.*, 2009). Food formulation quality usually depends on the processes involved from the farm to the final stage of consumption (Forsythe and Hayes, 1998). Personnel involved in handling such products need to be knowledgeable of the factors that contribute to production of good quality formulated nutritious flour meals that comply with the national and international standard requirements (FAO/WHO, 2004). It is equally important to identify the factors contributing to spoilage of flour meals in order to be able to institute control measures. Most relevant studies conducted in Tanzania were based on nutritional and feeding practice aspects such as assessment of Iron deficiency in complementary feeding (Mamiro *et al.*, 2004), assessment of mineral density in composite weaning products (Mosha and Vicent, 2005) and on nutritional status and feeding practices (Nyaruhucha *et al.*, 2006).

Currently there is an increase in production of different formulated infant flour meals in Tanzania due to increased demand of the products (FAO/WHO, 2004). These food products need to be produced in accordance with set requirements for health purposes of the consumers. This study was intended to provide important scientific information on microbiological status of formulated infant flour meals in the study area and elsewhere in the country so as to devise appropriate measures to minimize health problems to individuals consuming the products. The findings of this study would contribute to development of intervention strategies by various stakeholders to minimize microbiological contamination of the formulated infant flour meals and hence promoting health of target groups in the country.

1.3 Research Objectives

1.3.1 Overall objective

The overall objective of the study was to assess the microbiological quality of formulated infant flour meals available in local shops in Morogoro Municipality and their compliance to national quality standards.

1.3.2 Specific objectives

i. To determine levels of Total Plate Count (TPC)in the formulated infant flour meals available in local shops in Morogoro Municipality

ii. To determine levels of presumptive *Escherichia coli* contaminants in formulated infant flour meals available in local shops in Morogoro Municipality

iii. To determine levels of fungal (specifically moulds) contamination in formulated infant flour meals available in local shops in Morogoro Municipality.

1.4 Hypotheses

H_{O1}: There are no microbiological contaminations in formulated infant flour meals available in local shops found in Morogoro Municipality basing on Tanzanian standards.

H_{A1} There are microbiological contaminations in formulated infant flour meals available in local shops found in Morogoro Municipality basing on Tanzanian standards.

H_{O2} There is no significance difference in microbiological quality between brands of formulated infant flour meals taken for study

H_{A2} There is a significance difference in microbiological quality between brands of formulated infant flour meals taken for study

1.5 Limitation of the Study

The results of the study may be a source of development of constructive ideas for researchers and policy makers on nutritional foods. However, the study has some limitations including time and financial constraints in which case the researcher was not able to go further on isolation and identification of some important pathogenic microorganisms observed in formulated infant flour meals. The research was confined to specific objectives.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Background Information

Formulated complementary foods are infant foods formulated with appropriate nutritional quality for providing additional energy and nutrients which are either lacking or present in insufficient quantities in family foods and are suitable for use during the complementary feeding period of young children (Codex Alimentarius Commission, 2012). Based on food research conducted in various areas, health problem is one of the important targeted objectives to be minimized by formulated infant flour meals (Amankwah *et al.*, 2009). In Morogoro Municipality, formulated infant flour meals are mostly composed of such crops as finger millet, maize, rice, groundnuts, soybeans, wheat, sorghum and animal source. The formulation can involve one or several food items depending on the social need and standards requirements (Amankwah *et al.*, 2009).

Raw materials used to produce infant flour meals in most cases are locally cultivated within the countries of concern (Amankwah *et al.*, 2009). Infant flour meals may be formulated in order to target potential issues of health problems and market for generating household income. Preparation of the raw materials sometimes can involve such activities as fermentation and/or roasting before blending and milling of the raw materials. Fermentation process is well known to suit the socio-economic framework of developing countries as an affordable technology for the preservation and improvement of high carbohydrate based foods (FAO/WHO, 1997). The fermentation of cereals and cereal-based products in the traditional way depends largely on chance inoculation, involving mixed cultures of bacteria (Lactic acid bacteria), yeasts or both whereby in this process also untargeted microorganisms such as moulds (*Aspergillus* spp., *Penicillium* spp.,

Fusarium spp. and *Cladosporium* spp.) may interfere (Opere *et al.*, 2012). The common use of Lactic Acid Bacteria (LAB) for food fermentation in Africa is most probably due to the beneficial role of preservation, enhanced nutritional value, detoxification, rendering inedible foods edible and production of flavor varieties to the consumer (Opere *et al.*, 2012).

Worldwide, malnutrition have been observed to be a serious problem especially to developing countries for children with age between six to eighteen months, the period which need children to be supplemented with other food varieties than depending only on breast feeding (WHO, 1998; Bhandari *et al.*, 2004). It is reported that cereals and legumes contain nutrient potentials that could improve health of individuals if they will be prepared according to specified dietary requirements (Oguntona *et al.*, 1995; Fernandez *et al.*, 2002). Due to potentiality in important nutrients, the product becomes a good medium for microorganisms growth, some of which may spoil the product such that it will be unfit for human consumption or cause health problems to a consumer. Undernutrition to children under 5 years has been highlighted to the extent of tragedy (Badham, 2013). An estimation of 112 million children has been reported underweight and a further 178 million suffering from stunting (Badham, 2013). It has also been reported that poor breastfeeding practices and inadequate complementary feeding play a major role of undernutrition and cause over one-third of under-five children mortality (Badham, 2013).

The quality of flour depends on some processes involved in the raw materials as from the farm to the final stage of consumption by human or animal (Forsythe and Hayes, 1998). If good practices will not be done throughout or in some important stages during handling of raw materials or the flour itself, there are possibilities of producing contaminated infant flour meal.

2.2 Brands of Formulated Infant Flour

Worldwide there are various formulations of infant flour brands depending on the need and demand of the customers which differs from one country to another. The processing methods of the flour products differ in technologies used in developed countries and those from developing world where sophisticated technologies is employed compared to the later. Examples of brands from developed world includes; 'Gold medal all purpose flour' manufactured by Gold medal in the United States of America for health purposes, Semolina flour with a brand name of 'Bob's red mill' manufactured in Los Gatos, California, 'King Arthur Flour' produced in Clemson, south Carolina and 'Mr. Fresh ATTA' flour meal brand manufactured by Labh Singh and Sons company in India.

The known local brands which were available in the study area during the study were Shibe, Nadima, Wimbi, Imara, Power foods, Jazia meals, Robasa health foods, India mix, Wina products, Lusiga supplies, Sooji products from which samples were collected. Imported flour products in the study area were not found in the local shops and thus not included in the present study.

There are many entrepreneurs engaged in the business of self or group designed formulated infant flour meals for household economic purposes. During the study, it was not proved whether or not the products had ever been sent for quality assessment to responsible institutions like Tanzania bureau of standards (TBS) and Tanzania food and drugs authority (TFDA) and the product labels did not contain institutional logo for quality evidence. Sometimes producers sold their products directly to customers while some of the products had no brand name or trade name. However, there is another group of manufacturers who are well skilled on issues of production of acceptable quality of formulated infant flour. These manufacturers labeled their products with the required

information basing on our local requirements and sometimes they were provided with relevant training by responsible government institutions like Tanzania bureau of standards (TBS), Tanzania food and drugs authority (TFDA) and Tanzania food and nutrition centre (TFNC).

2.3 Microbiological Quality

Unsafe foods can cause food-borne diseases and have a negative health impacts to consumers. There have been many reported incidences of food-borne diseases and sometimes deaths in many countries in each year (FAO/WHO, 2004). Such problems may be underestimated since many countries lack strong surveillance and reporting systems (FAO/WHO, 2004). In developing countries due to poor reporting systems, some food-borne cases are not reported resulting to incorrect statistical figures for economics and disease incidences (FAO/WHO, 2004).

The significance of microbiological assessments is to ensure microbiological safety from raw materials to final food products. In May and June of 2011 an *E. coli* 0104:H4 outbreak was reported in Germany which was caused by contaminated imported sprouts and claimed to cause health problem to people who consumed the product (Rasko *et al.* 2011 and Scheutz *et al.*, 2011). Scientific research was conducted immediately to discover the source of the outbreak, to arrest the situation and stop the outbreak in the affected areas of Germany and strengthen their inspection and surveillance systems. This being a microbiological hazard, it can happen to any other food item if appropriate procedures of their production are not followed properly.

A research conducted in Pemba, had the objectives of studying the possibilities of presence of harmful microorganisms in infant flour meals (Kung'u *et al.*, 2009). One of

the outcomes was that food can get microbiological contamination between the time of preparation and consumption whereby aerobic bacteria, coliform bacteria and enterobacteriaceae were isolated (Kung'u *et al.*, 2009).

Various studies on the microbiological quality of flour meals have been conducted in western African countries of Ghana and Nigeria. A study conducted in Ghana on the weaning food formulations made up of fermented maize, rice, soybean and fishmeal showed the presence of bacteria, molds and yeasts at high numbers (Amankwah *et al.*, 2009). Other similar study by Ukwo *et al.* (2011) was conducted to assess microbiological quality of fresh juices and edible ice sold in Uyo Metropolis, Nigeria and reported high microbial contamination.

Another study was conducted to analyse multiple samples of the cereal based infant product revealed significant contamination with two spore-forming species, *Bacillus subtilis* and a strain of *Bacillus cereus*, with the latter being the most likely cause of the emetic food poisoning (Duc *et al.*, 2005). *Bacillus cereus* is one of the well-recognised food-poisoning organism that produces two kinds of illness: a diarrhoeal type and an emetic type (Granum and Lund, 1997; Kotiranta *et al.*, 2000; McKillip, 2000). The most common form is the diarrhoeal syndrome with an incubation period of 8–16 h before symptoms begin. The emetic toxin is thought to be the more dangerous of *B. cereus* toxins since it has caused fulminant liver failure, leading to death resulting from ingestion of large amounts of emetic toxin present in reheated food (Mahler *et al.*, 1997). In the potentially more dangerous emetic syndrome, vomiting can occur within 30 minutes of ingesting the preformed toxin, cereulide (Granum and Lund, 1997).

2.4 Quality of Packaging Materials for Flour Meals

Food packaging is important and the main purpose is to contain and protect the food product from deterioration resulting from chemical or physical induced changes, activities of microorganisms or pests (Forsythe and Hayes, 1998). Improper packaging of the products has also been implicated as a source of microbial contamination in food processing operations (Leistner, 1997). In the current study, infant flour products were observed to be packed in paper bags and Poly propylene bags. Whereas some of the products were packed in paper bags, Poly propylene bags or plastic films only, others were packed with both packaging material. Thus, foods were first packed in plastic films and then with paper bags on the outer part. It is recommended that packaging materials must be hygienic, odourless and inert and should not react with either contained food or surrounding atmosphere (Forsythe and Hayes, 1998).

2.5 Storage of Cereal Products

The levels of microorganism species of harvested cereal grains contain up to millions of bacteria and moulds per gram (Forsythe and Hayes, 1998).The low water activity of grains effectively inhibits the growth of most microorganisms provided proper storage conditions are satisfactory (Forsythe and Hayes, 1998). In moist conditions mould growth is likely and the most commonly isolated species are members of the genera *Penicillium, Aspergillus and Rhizopus* (Forsythe and Hayes, 1998). These are harmful mould species in cereals that may contaminate flour products if they are not excluded. From various findings, *Aspergillus flavus* has been well known to form Aflatoxins which are designated as B_1, B_2, G_1 and G_2 and are classed as heterocyclic compounds (Forsythe and Hayes, 1998). The toxins are heat resistant and only prolonged heating at 100^0C can destroy their potency (Forsythe and Hayes, 1998). The toxins have dangerous biological effects and that the main target organ is the liver where they can cause either tissue damage or tumour

(Forsythe and Hayes, 1998). Another common type of mould in cereals is *Penicillium* spp. which has been shown to form a number of toxins in foods, particularly rice. The toxins have been shown to cause liver cancer (*P. islandicum*), kidney damage (*P. citrinum)* and paralysis of central nervous system (*P. citreoviride*) (Forsythe and Hayes, 1998).

2.6 Compliance of Tested Samples to Microbiological Requirements of the Tanzania Standards

The aim of the study was to assess the microbiological quality of the formulated infant flour meals in relation to compliance with the set Tanzanian standards. Different categories of flour meals have different requirements of parameters to be tested. Tanzanian standard number 180 of 1983 processed cereal-based weaning foods specification) is used for the chemical and microbiological assessment of formulated nutritious flour meals. According to microbiological requirements for formulated infant flour meals, the total bacterial count per gram should not be more than 10^4 cfu/g, total coliforms should not be more than 10 MPN/g. However, the levels of *E. coli* and moulds have not been specified in this Tanzanian standard. According to World Food Program specification version 1 of 1999 for nutritious flour meals, the requirements for total plate count should not be more than 10^5cfu/g, total coliforms not more than 10^2 MPN/g, while requirements for *E. coli* and moulds have not been indicated. Since the local standards are silent on the requirements for *E. coli* and Fungi, there is a need for responsible parties in reviewing the respective standards because in these groups of microorganisms there are important pathogenic species which can cause health problems to consumers.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Description of the Study Area

Morogoro Municipality is located in Morogoro Region at an altitude ranging from 500-600 m above sea level, longitude 37°-39°E and latitude 6°-5° S. Morogoro Municipality is estimated to have a population of 315,866 (National Bureau of Statistics (NBS) of Tanzania, 2012). It has a mixture of warm and cool temperature ranging between 27°C to 33.7°C in the dry/ warm seasons and 14.2°C to 21.7°C in cold/wet season. According to Tanzania Meteorological Agency (TMA), the Municipality of Morogoro experiences a sub-humid tropical climate with a bimodal rainfall pattern which are characterized by two rainfall seasons in a year with a dry season separating the short rains (October to December) and long rains (from March to May/June).

3.2 Study Design

In this study, a cross sectional study design was employed which allowed samples to be collected at one time from the sources. A survey was first conducted to identify different brands of infant flour formulations with at least two food items blended to form the final intended product. The study units involved formulated infant flour meals packed in 1 kg bag each and were drawn from local shops available in Morogoro Municipality. The study samples were qualified based on their batch numbers and expiry date of within six months of the study. In this case, each group of brand selected for inclusion in a study had to bear same batch numbers. A simple random sampling was conducted in which case each brand had an equal chance of being sampled. Local shops from which samples were drawn were identified by researcher assigned numbers (1 to 16) during the study. Also the samples

drawn were identified by letters $A_{1\text{-}15}$, $B_{1\text{-}15}$, $C_{1\text{-}15}$, $D_{1\text{-}15}$, $E_{1\text{-}15}$, $F_{1\text{-}15}$ and $G_{1\text{-}15}$. Sample identification, locations of local shops where samples were drawn and sample ingredients are detailed in Table1.

3.3 Determination of the Sample Size

Sample size for the study was determined through the approach based on precision rate and confidence level. The following formula for known population was employed to determine the sample size (Kothari, 2004):

$$n = \frac{N \times Z^2 \times SD^2}{(N-1) \times e^2 + Z^2 \times SD^2} \quad \text{.....(1)}$$

Where;

n = estimated sample size

Z = t value for an expected confidence level (1.96)

SD = expected standard deviation (to be 0.1)

e = selected accepted error or precision (as 0.05) and

N = Population size (in this case N was 12 brands)

The numbers of brands to be included in the study were calculated to be 7 out of 12 brands. The formula for unknown population was applied for determining the number of flour bags to be drawn in each of the seven calculated brands from the local shops. This is because it is not easy to get the total quantity of the flour produced on a particular day from the surveyed local shops. The formula for unknown population applied was as follows (Kothari, 2004):

$$n = \frac{Z^2 \times SD^2}{e^2} \quad \text{.....(2)}$$

Where;

n = estimated sample

Z = t value for an expected confidence level (1.96)

SD = expected standard deviation (to be 0.1)

e = selected accepted error or precision (as 0.05)

Step 1: Determination of sample for flour brands to be involved

Sample (n) = $(12 \times 1.96^2 \times 0.1^2)/ [(12-1) \times 0.05^2 + (1.96^2 \times 0.1^2)] = 7$ brands

Step 2: Determination of sample for flour bags to be drawn from the local shops

Sample (n) = $(1.96^2 \times 0.1^2)/ \times 0.05^2 = 15$ bags

Step 3: Sample size of flour bags = 7 brands x 15 bags =105 bags

The following table illustrates sampling mechanism employed in the study;

Table 1: Infant flour sampling mechanism

	Samples							
Sampling locations	**A_{1-15}**	**B_{1-15}**	**C_{1-15}**	**D_{1-15}**	**E_{1-15}**	**F_{1-15}**	**G_{1-15}**	**Total**
1	1	2	0	1	2	0	1	7
2	0	1	2	0	1	2	1	7
3	2	0	1	1	0	0	1	5
4	1	0	1	1	2	1	1	7
5	1	1	1	0	0	1	1	5
6	0	1	2	1	2	0	0	6
7	1	1	0	1	1	0	2	6
8	0	1	2	0	2	1	1	7
9	1	2	1	1	0	2	0	7
10	2	1	1	2	1	0	1	8
11	0	0	2	1	0	1	2	6
12	2	1	0	0	0	2	1	6
13	1	1	2	1	2	0	1	8
14	0	2	0	1	0	2	2	7
15	2	0	0	1	0	1	0	4
16	1	1	0	3	2	2	0	9
Total	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**105**

Table 1. Sampling mechanism used in order to get samples of formulated infant flour bags of 1kg each.

3.4 Microbiological Analysis

The microbiological analysis was done as from 22 October, 2012 to 11 January, 2013 in the Microbiology laboratory at the Faculty of Veterinary Medicine and in the Plant Pathology laboratory in the Department of Soil Science at Sokoine University of Agriculture.

3.5 Ingredients and Preparation of Media Used in the Study

3.5.1 Standard plate count agar ingredients (in g/l) and preparation

Enzymatic digestion of casein	5.0 g
Yeast extract	2.5 g
Glucose, anhydrous ($C_6H_{12}O_6$)	1.0 g
Agar	15.0 g
Distilled water	1000 ml

The media used was ready made as powdery form and prepared as per manufactures instructions by suspending 23.5g of the dehydrated media in one litre of distilled water and sterilized by autoclaving at 121°C for 15 minutes and cooled to 45°C in the water bath.

3.5.2 Buffered peptone water ingredients (in g/l) and preparation

Peptone	10.0 g
Sodium chloride	5.0 g
Disodium phosphate	3.5 g
Potassium dihydrogen phosphate	1.5 g
Distilled water	1000 ml

The media used was ready made as powdery form and prepared as per manufactures instructions by suspending 20g of the dehydrated media in one litre of distilled water and sterilized by autoclaving at 121°C for 15 minutes and cooled.

3.5.3 Lauryl tryptose broth ingredients (in g/l) and preparation

Tryptose	20.0 g
Lactose	5.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.75 g
Potassium dihydrogen phosphate	2.75 g
Sodium lauryl sulphate	0.1 g
Distilled water	1000 ml

The media used was ready made as powdery form and prepared as per manufactures instructions by suspending 35.6g of the dehydrated media in one litre of distilled water and sterilized by autoclaving at 121°C for 15 minutes and cooled

3.5.4 Malt extract agar ingredients (in g/l) and preparation

Maltose	12.75 g
Dextrin	2.75 g
Peptone	0.78 g
Agar	15.0 g
Distilled water	1000 ml

The media used was ready made as powdery form and prepared as per manufactures instructions by suspending 31.3 g of the dehydrated media in one litre of distilled water, adding 0.1 g of Chloramphenicol to suppress bacterial growth ,sterilized by autoclaving at 115°C for 10 minutes and cooled to 45°C in the waterbath.

3.5.5 EC broth ingredients (in g/l) and preparation

Tryptone	20.0 g
Lactose	5.0 g
Bile salts No. 3	1.5 g
Di-potassium phosphate	4.0 g
Mono-potassium phosphate	1.5 g
Sodium chloride	5.0 g
Distilled water	1000 ml

The media used was ready made as powdery form and prepared as per manufactures instructions by suspending 37g of the dehydrated media in one litre of distilled water and sterilized by autoclaving at 121°C for 15 minutes and cooled

3.5.6 Tryptone water ingredients (in g/l) and preparation

Enzymatic digest of casein	10.0 g
Sodium chloride	5.0 g
Distilled water	1000 ml

The media used was ready made as powdery form and prepared as per manufactures instructions by suspending 15g of the dehydrated media in one litre of distilled water and sterilized by autoclaving at 121°C for 15 minutes and cooled

3.5.7 Kovacs/Indole reagent ingredients

4-Dimethylaminobenzaldehyde	5.0 g
2-Methylbutan-1-ol	75.0 ml
Hydrochloric acid (1.18 d/ml)	25.0 ml

The reagent was ready made in a liquid form.

3.6 Laboratory Work

3.6.1 Determination of levels of microbial contaminants using total plate count (TPC) method

Determination of levels of microorganisms using TPC method in the formulated infant flour meals was done according to the national standard number 118 of 2007 (TZS 118, 2007) which is equivalent to ISO 4833, 2003. The standard defines the term microorganism as the combination of bacteria and fungi. This standard details the enumeration of bacterial and fungal contaminants using total plate count technique at 30°C for 72 h in order to get an estimated number of colony forming units per each gram (cfu/g) of the flour meals collected for analysis.

3.6.1.1 Protocol on the determination of levels of TPC contaminants

The protocol for determination of TPC is summarized in Figure 1. Briefly, preparation of initial suspension of infant flour meal was done aseptically by taking 25 g of the flour sample into 225 ml of sterile buffered peptone water (Oxoid CM0509 1049194 England) making 10^{-1} dilution factor. Adequate tubes containing 9 ml of diluents of sterile 0.1% buffered peptone water were prepared and labeled as 10^{-1} to 10^{-6}. The first prepared diluent was inoculated with 1 ml from the initial suspension (making 10^{-2} dilution) and vortexed, 1 ml from the first tube was inoculated to the second diluent and the process was repeated until the last tube that was diluted to 10^{-6}. Sterile Petri dishes were prepared and labeled for easy identification of the sample under test. From the initial suspension using sterile micropipette, 1 ml was inoculated into the first sterile Petri dish labeled 10^{-1}. From the first diluents (10^{-2}) 1 ml was inoculated to the next sterile Petri dish labeled 10^{-2} and similar process was done to the rest of the Petri dishes. About 15 ml of the sterile standard plate count agar medium (Oxoid CM0463B 1146440 England) previously sterilized in the

autoclave set at 121^{0}C for 15 minutes then cooled and maintained at 45±1^{0}C in a water bath was aseptically poured into the inoculated Petri dishes in the clean biosafety cabinet. The inocula were gently mixed and allowed to solidify for about 10 minutes. The Petri dishes with solidified media were incubated at 30^{0}C for 72 h. Controls of standard plate count agar blank Petri dishes and sterility test for the diluents were done for each batch of the analysis and incubated in the same incubator. The aim of this testing method was to evaluate the load of microorganisms in flour samples grown in solid media (Plate Count Agar) by direct observation in which case each colony was counted as a single unit (colony forming unit-CFU) to estimate the total level of contamination.

3.6.1.2 Controls for TPC

- Blank Petri dishes were prepared by pouring plate count agar in sterile Petri dish, left to solidify and incubated together with TPC Petri dish for every batch of analysis.
- Sterility testing was done by inoculating 1 ml of sterile diluents of 0.1 % BPW followed by pouring PCA in a sterile Petri dish, left to solidify and incubated at 30°C for 72 h.
- Working environment was monitored by using environmental monitoring Petri dishes. These were prepared by pouring about 15 ml of PCA into sterile Petri dishes and then left to solidify. After solidification the Petri dishes were placed on working areas such as in biosafety cabinet, weighing area and in the incubator while open for 10 minutes and collected for incubation at 30^{0}C for 72 h. This process was done for every batch of the food samples. Also blank Petri dishes, solidified petri dishes for sterility test and environmental monitoring Petri dishes were also incubated at 30^{0}C for 72 h.

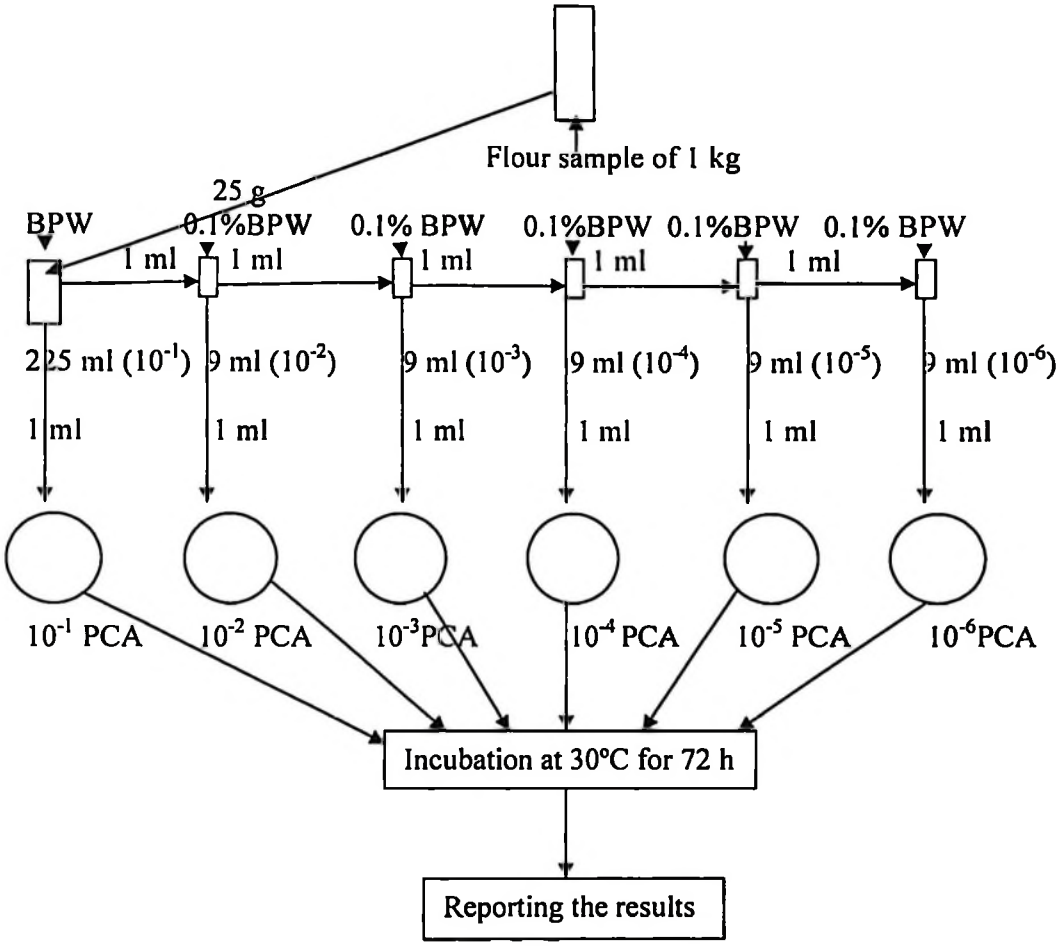

Figure 1: Schematic drawing for the determination of Total Plate Count levels in infant flour meal

3.6.2 Determination of levels of *Escherichia coli* contaminations

Determination of levels of *E. coli* contaminations in the formulated infant flour meals was done according to Tanzanian local standard number 731 of 2007 (TZS 731, 2007). This standard details on the horizontal method for the detection and enumeration of presumptive *E. coli* using most probable number technique. The standard is equivalent to ISO 7251, 2005. Evaluation of *E. coli* levels was achieved through steps as detailed in the protocol below:

3.6.2.1 Protocol on the assessment of levels of *Escherichia coli* (TZS 731, 2007)

The protocol for determination of *E. coli* is summarized in Figure 2. Briefly, a sample portion of 25 g was added into 225 ml of sterile non selective medium buffered peptone water (making 10^{-1} dilution) and mixed thoroughly. Adequate tubes containing 9 ml of diluents of sterile 0.1% buffered peptone water (Oxoid CM0509 1049194 England) were prepared and labeled for sample identification (A to G) and dilution factor (10^{-2} to 10^{-5}). The first prepared diluent was inoculated with 1 ml of the initial suspension and vortexed (making 10^{-2} dilution). Again 1 ml from the first tube of 10^{-2} dilution was inoculated to the next diluents and the same process was done until the last 10^{-5} dilution. Three universal bottles of double strength (containing 10 ml) and fifteen single strength test tubes (all batches containing Durham tubes inside) in groups of three of lauryl sulphate tryptose broth (LST) were prepared for each sample. Liquid selective media of lauryl sulphate tryptose broth (Oxoid CM0451b 1228172 England) was inoculated with 10 ml aliquots from the initial suspension into three double strength universal bottles and 1 ml aliquots into the next three single strength tubes (each containing 9 ml of LST). Again 1 ml aliquots from 10^{-2} diluent were inoculated into the other three test tubes and similar process was done to the rest of the diluents and LST test tubes until 10^{-5} dilution. The inoculated universal bottles and test tubes were gently mixed and incubated at 37^{0}C for 48 h. After 48 h the tubes were examined for gas production, opacity and cloudiness. All the tubes which showed opacity, cloudiness and gas production were sub-cultured into test tubes containing 10 ml sterile EC-broth (containing inverted Durham tubes) using sterile wire loops and incubated at 44^{0}C for 48 h and examined for gas production. All the tubes of EC broth (Liofilchem 051410208 Italy) which gave rise to gas production were again sub cultured into tubes each containing sterile 10 ml indole-free peptone water (Oxoid CM0087B 977649England) and incubated at 44^{0}C for 48 h. Finally all the tubes were

examined for indole production. For each dilution, the number of positive results for double strength and for single strength medium was enumerated using MPN tables. Expectations of this test method were to detect and enumerate levels of *E. coli* strains able to grow at 44^{0}C. In this case only the reaction between Kovacs reagent (Scharlau REooo7G100 12887701 Spain) and degraded tryptophan giving rise to indole production (a red colour in the alcoholic phase) indicated presence of *E. coli* strains in the sample.

3.6.2.2 Controls for *Escherichia coli*

- A negative control used was a strain of *Staphylococcus aureus* ATCC 6538. The strain was inoculated in parallel with all analysis stages into sterile LST, EC broth and TW until confirmation of *Escherichia coli* (indole testing)
- At this stage, positive control was *Escherichia coli* strain with ATCC number 8739. The strain was inoculated in parallel with all analysis stages into sterile LST, EC broth and TW until confirmation of. *Escherichia coli* (indole testing)
- Also blanks of LST, EC broth and TW were incubated for evaluating their sterility as blank controls.

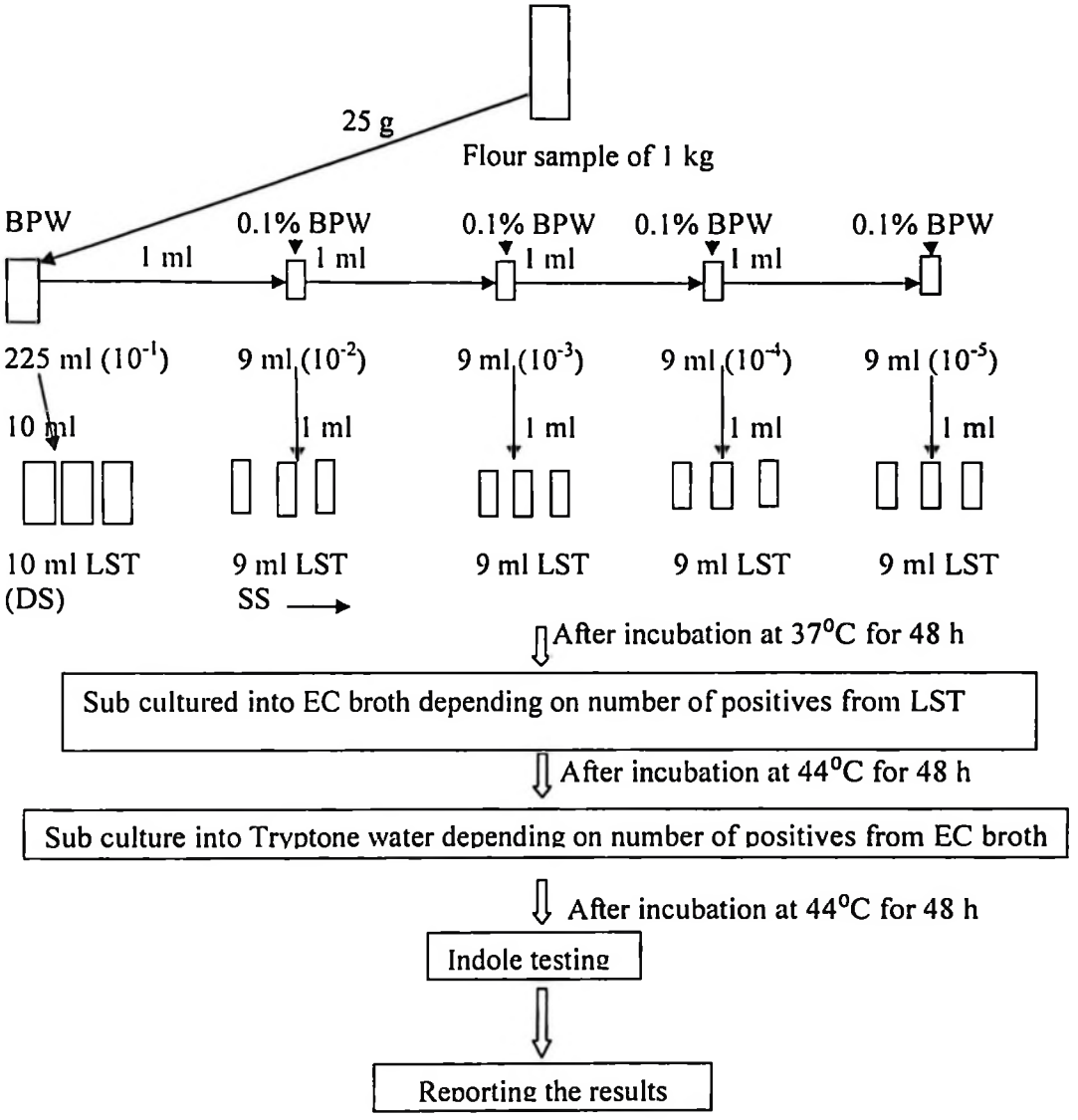

Figure 2: Schematic drawing for determination of *Escherichia coli* levels in infant flour meal

3.6.3 Determination of levels of fungal contaminants

Determination of levels of fungal contaminants in the formulated infant flour meals was done according to the International standard number 7954 of 1987 (ISO 7954, 1987). This standard details on the general guidance for enumeration of yeasts and molds using colony count technique at 25°C. The levels of molds were evaluated using the following protocol;

3.6.3.1 Protocol for the determination of levels of fungi (ISO 7954, 1987)

The protocol for determination of fungal contaminants is summarized in Figure 3. Briefly, a preparation of initial suspension in which 25 g of the flour sample was added into 225 ml of sterile buffered peptone water making 10^{-1} dilution factor. Adequate test tubes containing 9 ml of diluents of 0.1% buffered peptone water were prepared. Each sample used five test tubes of diluents. The tubes were labeled for sample identification (A to G) and dilution factor (10^{-2} to 10^{-6}). The first prepared diluent was inoculated with 1 ml from the initial suspension (making 10^{-2} dilution) and vortexed. Another 1 ml from 10^{-2} dilution was inoculated into the next diluents (making 10^{-3} dilution). The same process was done to the rest of the test tubes until 10^{-6} dilution. Sterile Petri dishes were prepared and labeled for sample identification (A to G), dilution factor (10^{-1} to 10^{-6}) and date of analysis. One ml from initial suspension was inoculated into 10^{-1} labeled sterile Petri dish, 1 ml from the first diluent (10^{-2}) was inoculated into the next Petri dish and the same process done to the rest of Petri dishes until 10^{-6} diluent. About 15 ml of the sterile Malt extract agar medium (Liofilchem 072412205 Italy) previously melted and maintained at 45°C in a water bath was poured into the inoculated Petri dishes. The inocula were gently mixed and allowed to solidify for about 10 minutes. The solidified Petri dishes were incubated at 25°C for 5 days. Fungal colonies were counted and reported as colony forming units per gram of infant flour meal. Microscopic examination was carried out to distinguish different morphological characteristics of molds. Figure 5 to 7 shows different species of moulds that were observed using a light microscope.

3.6.3.2 Controls for Fungi analysis

- Blank Petri dishes were prepared by pouring malt extract agar medium in sterile Petri dish, left to solidify and incubated together with test Petri dishes for every batch of analysis.
- Sterility testing was done by inoculating 1 ml of sterile diluents of 0.1 % BPW followed by pouring malt extract agar medium in a sterile Petri dish, left to solidify and incubated at 25°C for 5 days.
- Environmental monitoring Petri dishes were prepared by pouring about 15 ml of PCA into sterile Petri dishes and then left to solidify. After solidification the Petri dishes were placed in appropriate areas namely, the Biosafety cabinet, sample weighing area and in the incubator. Plates were left open for 10 minutes and then collected and incubated at 30°C for 72 h. This process was done for every batch of laboratory microbiological analysis.

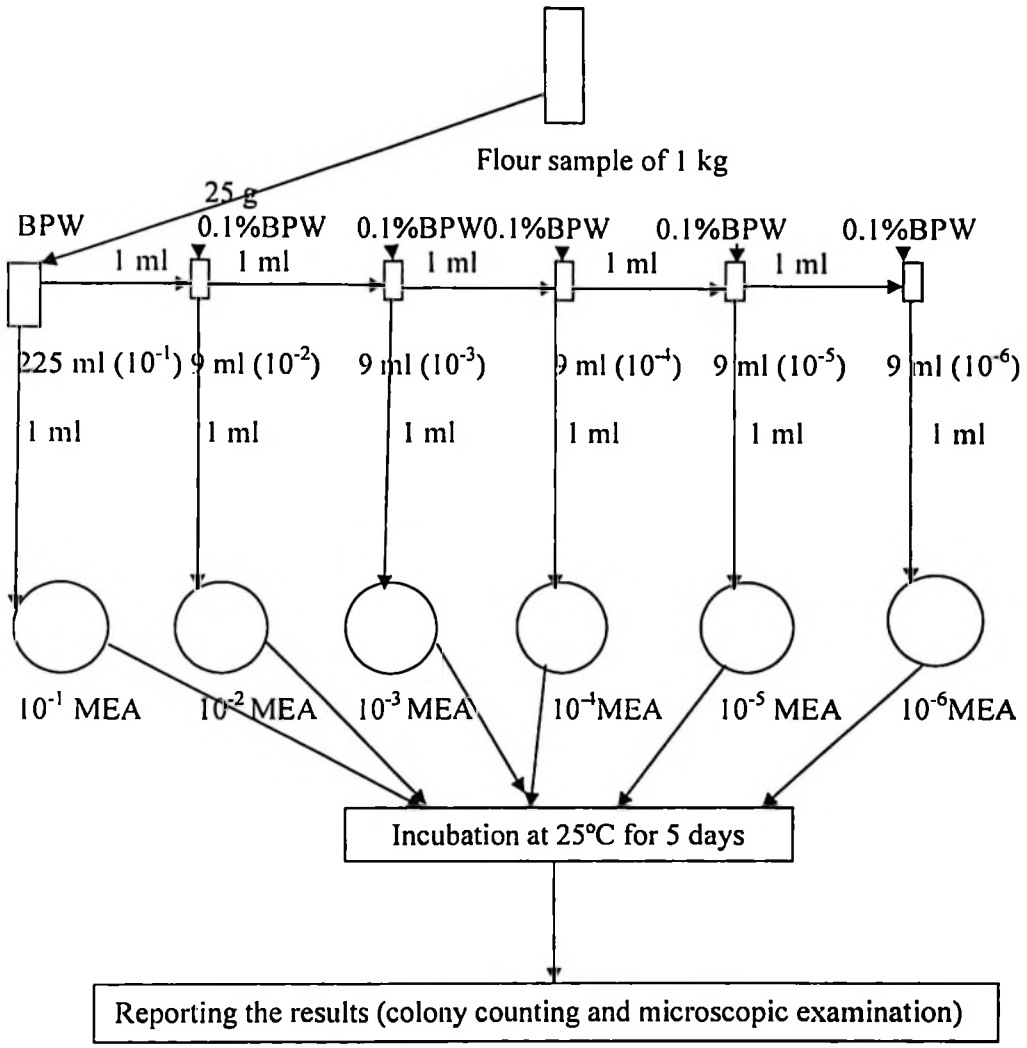

Figure 3: Schematic drawing for determination of fungal contaminants in infant flour meal

3.6.3.3 Fungal microscopic examination

Microscopic examination of fungal growth (specifically moulds) was carried out to assess the presence of common moulds like *Aspergillus* spp. and *Penicillium* spp. The process was first done by preparing microscope slides. Using an inoculating needle a small portion of each fungal growth was picked from the Petri dishes and placed on separate slides containing a drop of Lacto Pheno Cotton Blue (LPCB) stain, followed by teasing and

emulsification with inoculating needle. A cover slip was then placed on the slide and then microscopic examination was done under a low (40x) and high power (100x) objectives using immersion oil for the latter. Photographs for observed moulds were taken using a special camera at 100x magnification.

Table 2: Expected microscopic appearance of target mould species

Fungi (Moulds)	Microscopic appearance
Aspergillus species	Hyaline and septate hyphae, unbranched conidiophores with foot cell, large vesicle at tip which supports short, flask-shaped phialides (sterigmata) and each phialide produces chains of round phialoconidia
Penicillium species	Septate and hyaline hyphae, simple or branched conidiophores, phialides are usually bunched in brush like clusters called 'conidia' at the tips of conidiophores. Conidia are single celled, round or ovoid, hyaline or pigmented, rough or smooth walled and in chains

3.7 Data Analysis

The primary data for the variables were obtained from microbiological laboratory analysis of the sampled infant flour meals. Research data from other related studies were used for comparisons. Research data were analysed to get means and standard deviations using Microsoft office excel 2007 and statistical techniques of analysis of variance (ANOVA) in Open Epi version 2.3.1 programme was used to calculate p-values for comparing the variations of microbiological qualities between the analysed infant flour brands.

3.8 Confidentiality

Since the study was based on formulated infant flour meals found in the local shops in Morogoro Municipality, confidentiality was guaranteed by identifying samples selected

for the study using letters $A_{1\text{-}15}$, $B_{1\text{-}15}$, $C_{1\text{-}15}$, $D_{1\text{-}15}$, $E_{1\text{-}15}$, $F_{1\text{-}15}$ and $G_{1\text{-}15}$ in order to avoid conflicts of interest between the manufacturers of the products, authorities responsible for similar activity and the researcher. The researcher retained the corresponding product brand names in relation to assigned identification letters.

CHAPTER FOUR

4.0 RESULTS

A total 105 samples of formulated infant flour meals were collected from 16 shops. The shops were identified during the survey basing on the availability of infant flour meals qualified for the study. The details of the nature of samples and the laboratory results are hereby detailed in the subsequent sections of the dissertation.

4.1 Composition of the Sampled Formulated Infant Flour Meals

The formulated infant flour meals used in this research had various food ingredients as indicated in the Table 3. The ingredients were indicated on corresponding label attached on packaging material of each sample. There were no details of percentage for each component used in flour production. This could be due to the fact that entrepreneurs do not want to expose their formula used in production and therefore remains a secret so that it will not be copied by their competitors.

Table 3: Study sample compositions

S/N	Sample ID	Local shop sampled	Sample ingredients
1	A_{1-15}	1, 3,4,5,7,9,10,12,13,15 and 16	Rice, Maize, Soybean, Groundnuts and Finger millet
2	B_{1-15}	1,2,5,6,7,8,9,10,12,13,14 and 16	Rice, Maize, Soybean, Groundnuts, Sorghum, Wheat and Finger millet
3	C_{1-15}	2, 3,4,5,6,8,9,10,11 and 13	Rice, Maize, Soybean, Groundnuts, Sorghum and Finger millet
4	D_{1-15}	1,3,4,6,7,9,10,11,13,14,15 and 16	Rice, Maize, Soybean, Groundnuts and Finger Millet
5	E_{1-15}	1,2,4,6,7,8,10,13 and 16	Fish, Rice, Maize, Carrot, Soybean, Groundnuts, Vegetable and Wheat
6	F_{1-15}	2,4,5,8,9,11,12,14,15 and 16	Rice, Maize, Soya bean, Groundnuts and Finger millet
7	G_{1-15}	1,2,3,4,5,7,8,10,11,12,13 and 14	Maize, Soybean, Sorghum, Pearl millet and Finger millet

Table 3 details the different samples included in the study, locations where they were sourced as identified by numbers in Morogoro Municipality and ingredients used to manufacture the formulated infant flour meals. The reported food ingredients as observed from the package labels were not proved/analysed by food type or nutrient type. However, food composition may have influence on type of microorganism population.

4.2 Total Plate Count Estimation

The data are reported as means of fifteen replicates from fifteen analysed flour bags of the same manufacturing date and batch number and the corresponding standard deviation. Table 4 present the results of TPC from the present study and compared to Tanzania standard 180, 1983.

Table 4: Total plate count results against Tanzania standard number 180, 1983

S/N	Sample ID number	TPC (cfu/g) (This study)	TZS 180, 1983 requirements (cfu/g), Maximum
1	A_{1-15}	$(2.67 \pm 0.29) \times 10^5$	10^4
2	B_{1-15}	$(3.43 \pm 0.71) \times 10^4$	10^4
3	C_{1-15}	$(2.89 \pm 0.26) \times 10^5$	10^4
4	D_{1-15}	$(4.09 \pm 1.77) \times 10^5$	10^4
5	E_{1-15}	$(1.16 \pm 0.14) \times 10^5$	10^4
6	F_{1-15}	$(9.74 \pm 1.73) \times 10^4$	10^4
7	G_{1-15}	$(1.73 \pm 0.20) \times 10^5$	10^4

TPC results are represented as means and standard deviation of formulated infant flour meals. Letters A_{1-15}, B_{1-15}, C_{1-15}, D_{1-15}, E_{1-15}, F_{1-15} and G_{1-15} are researcher's sample identifications.

Results for TPC indicate high counts that do not comply with local requirements. According to the respective Tanzanian standard (TZS 180, 1983) which provides chemical and microbiological specifications for processed cereal based weaning foods, the requirement for TPC is that it shall not exceed 10^4 cfu/g of the analysed samples. Basing on TPC results none of the seven samples complied with the requirements of Tanzanian local standard of Tanzania, TZS 180, 1983. Results for TPC ranged from (3.43 ± 0.71) $x10^4$cfu/g to (4.09 ± 1.77) $x10^5$ cfu/g. Sample $D_{1\text{-}15}$ has shown to have highest TPC of all samples and sample $B_{1\text{-}15}$ shown lowest counts of all samples taken for the study. The results of TPC ranking order from the highest to lowest as per Table 4 are $D_{1\text{-}15}$, $C_{1\text{-}15}$, $A_{1\text{-}15}$, $G_{1\text{-}15}$, $E_{1\text{-}15}$, $F_{1\text{-}15}$ and $B_{1\text{-}15}$. These results of TPC thus show the level of hygiene of the infant flour meals from respective manufacturers.

According to WFP Version 1:1999 infant flour Specification, the requirements are that TPC shall not exceed 10^5cfu/g. When this standard is used to qualify the infant flour products analysed in the study, only two samples ($B_{1\text{-}15}$ and $F_{1\text{-}15}$) with TPC counts of (3.43 ± 0.71) $x10^4$ and (9.74 ± 1.73) $x10^4$ respectively conforms to WFP Version 1:1999 Nutritious flour Specification. Normally infant flour meals are tested basing on our national standards. Total Plate Count is mostly used as an indicator of hygienic standard or potential health hazard (Harrigan, 1998). Fig. 4 is a photograph of one of the batches of TPC technique for sample B taken at countable dilutions whereby Plate count agar was used for growing microorganisms.

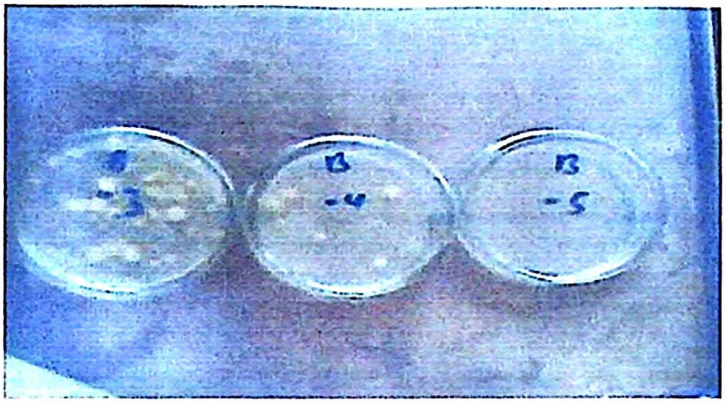

Figure 4: Showing a batch of TPC Petri dishes containing microorganisms colonies From sampled infant flour meals from Morogoro Municipality

4.3 *Escherichia coli* Contamination

Levels of *E. coli* contamination were evaluated in the formulated infant flour meals using MPN technique which utilizes multiple tubes to get an estimate number of *E. coli*. The data are reported as means of fifteen replicates from fifteen analysed flour bags of the same manufacturing date and batch number and the corresponding standard deviation. Table 5 details the results of *E. coli* MPN.

Table 5: *E. coli* MPN results against the Tanzania standard number 180, 1993

S/N	Sample ID number	*E. coli* (MPN/g) (This study)	TZS 180, 1983 requirements
1	$A_{1\text{-}15}$	6 ± 3	NI
2	$B_{1\text{-}15}$	1 ± 1	NI
3	$C_{1\text{-}15}$	1 ± 1	NI
4	$D_{1\text{-}15}$	71 ± 58	NI
5	$E_{1\text{-}15}$	5 ± 3	NI
6	$F_{1\text{-}15}$	0	NI
7	$G_{1\text{-}15}$	0	NI

E. coli results are represented as means and standard deviation for infant flour meal

Note: NI means not indicated in the standard

Table 5 shows results of *E. coli* contaminants in analysed infant flour meals. Note that sample D_{1-15} has 71 MPN/g which is the highest MPN of all seven samples subjected in the study while *E. coli* was not detected in samples F_{1-15} and G_{1-15}. Sample B_{1-15} and C_{1-15} had similar *E. coli* results of 1 MPN/g each whereby sample A_{1-15} and E_{1-15} had closely similar results of 6 MPN/g and 5 MPN/g respectively. Tanzanian standard number 180 of 1983 which provides chemical and microbiological specifications for processed cereal based weaning foods does not indicate requirements for *Escherichia coli* but indicates requirements for total coliforms which are suppose not to exceed 10 MPN/g.

4.4 Fungal Contaminants

Fungal data are reported as means of cfu/g of infant flour meal and their corresponding standard deviation. Table 6 details the results of Fungi.

Table 6: Fungi results against Tanzania standard 180, 1983

S/N	Sample ID	Yeasts/Moulds (cfu/g) (This study)	TZS 180, 1983 Requirement
1	A_{1-15}	$(2.32 \pm 0.60) \times 10^3$	NI
2	B_{1-15}	$(1.22 \pm 0.11) \times 10^4$	NI
3	C_{1-15}	$(4.67 \pm 0.93) \times 10^3$	NI
4	D_{1-15}	$(4.13 \pm 0.63) \times 10^4$	NI
5	E_{1-15}	$(8.01 \pm 1.10) \times 10^4$	NI
6	F_{1-15}	$(1.92 \pm 0.20) \times 10^4$	NI
7	G_{1-15}	$(4.16 \pm 0.83) \times 10^4$	NI

Note: NI means not indicated in the standard

Fungal results as shown in Table 6 indicate high counts ranging from $(2.32 \pm 0.60) \times 10^3$cfu/g to $(8.01 \pm 1.10) \times 10^4$cfu/g for sample A_{1-15} and E_{1-15}, respectively. Tanzanian standard number 180 of 1983 which provides chemical and microbiological specifications

for processed cereal based weaning foods does not indicate requirements for fungi, specifically yeasts and moulds. The fungal colonies were observed to have different morphological characteristics. Microscopic examination was used to identify some few common mould species. *Aspergillus* spp., *Cladosporium* spp. and *Penicillium* spp. were identified using microscopic technique in samples $A_{1\text{-}15}$, $B_{1\text{-}15}$, $D_{1\text{-}15}$ and $F_{1\text{-}15}$ only. Studied colonial appearance of target species were noted as follows: *Aspergillus* spp were green, brown and black; *Penicillium* spp were green, grey-green and blue-green and *Cladosporium* spp were dark-green and black.

The data in Table 6 shows the levels of fungal contaminants whereby different brands had different cfu/g with brand $E_{1\text{-}15}$ having the highest and brand $A_{1\text{-}15}$ having the lowest fungal counts. *Aspergillus* spp. were found in samples $A_{1,3,4}$ and $B_{2,4}$; *Penicillium* spp. in samples $A_{1,5}$ and $F_{7,11}$ and *Cladosporium* spp. in samples $A_{5,8,}$ $B_{1,4,6}$ and D_3 as in Fig. 5 to 7. The photographs were taken using a light microscope at 100x magnification.

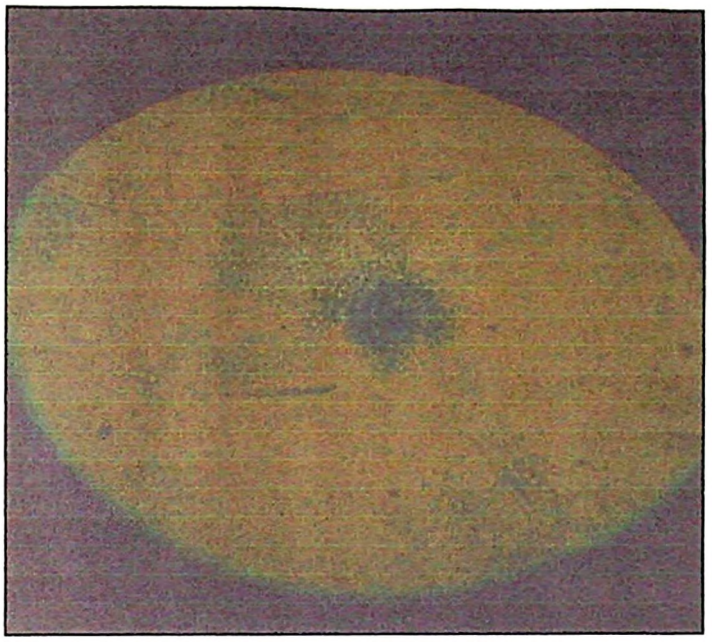

Figure 5: Showing *Aspergillus* specie on slides under microscopic observation which were found in sampled infant flour meals from Morogoro Municipality

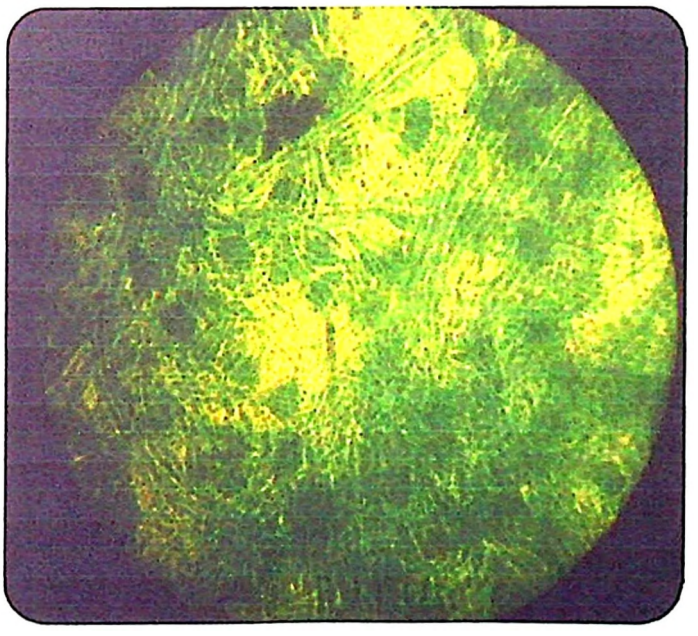

Figure 6: Showing *Penicillium* species on slides under microscopic observation which were found in sampled infant flour meals from Morogoro Municipality

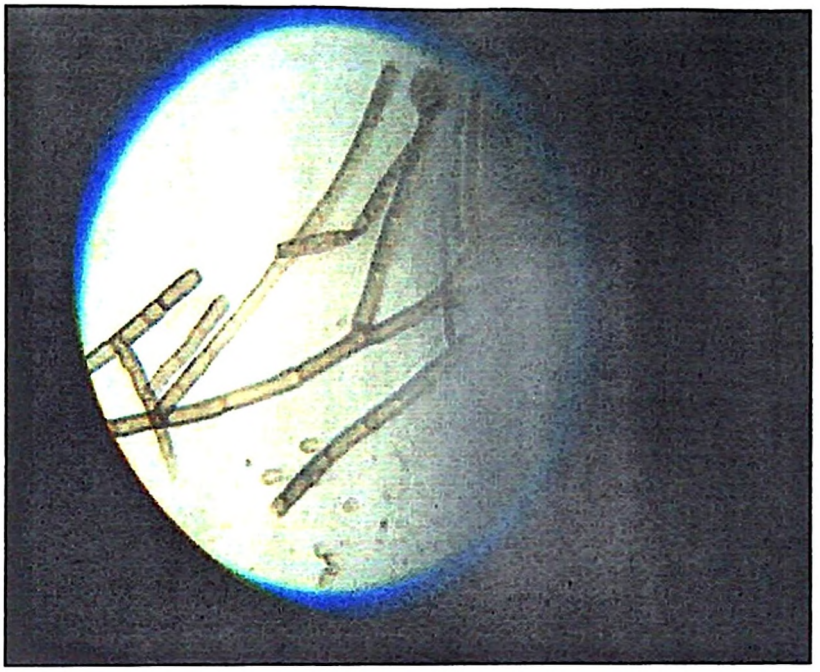

Figure 7: *Cladosporium* species on slides under microscopic observation which were found in sampled infant flour meals from Morogoro Municipality

4.5 Microbiological Quality Comparison between Manufacturers

The results from the study were converted into log base ten (Log_{10}) for all three parameters and subjected into ANOVA in order to evaluate if there is any significant difference in microbiological quality between brands of infant flour meals collected for the study. The null hypothesis on this particular study can be stated as there is no significant difference in microbiological quality between brands of infant flour samples collected for microbiological analysis, whereby the reverse of the statement is considered as an alternative hypothesis.

Table 7: Calculated F-statistics and p-values for TPC, *E. coli* and Fungi

Sample ID	TPC (log cfu/g) (This study)	*E. coli* (log MPN/g) (This study)	Yeast/Mould (log cfu/g) (This study)
A_{1-15}	5.42 ± 0.05	0.72 ± 0.21	3.35 ± 0.11
B_{1-15}	4.53 ± 0.09	-0.45 ± 0.54	4.08 ± 0.04
C_{1-15}	5.46 ± 0.04	-0.37 ± 0.50	3.66 ± 0.09
D_{1-15}	5.58 ± 0.17	1.68 ± 0.43	4.61 ± 0.07
E_{1-15}	5.06 ± 0.05	0.69 ± 0.19	4.90 ± 0.06
F_{1-15}	4.98 ± 0.08	0.0	4.28 ± 0.05
G_{1-15}	5.24 ± 0.05	0.0	4.61 ± 0.09
F_C	258.82	74.20	795.82
P-value	9.0×10^{-58}	3.12×10^{-34}	9.1×10^{-81}

Results in this table are represented as means and standard deviation in a log form.

Results in Table 7 show a summary of ANOVA for tested parameters of TPC, *E. coli* and Fungi whereby the original data in a power form were first converted into logarithmic form and calculated for means and corresponding standard deviations. Results show that there is a significant difference in microbiological quality between brands of infant flour meals since p-values for all three parameters tested (TPC, *E. coli* and Fungi) are less than an estimated error ($p<0.05$) meaning that the null hypothesis is rejected in favour of the alternative hypothesis. ANOVA calculations for the three parameters are available in the appendices page (appendix 1, 2 and 3). The infant flour brands studied were not consistent in terms of microbiological quality performance most probably because they are produced by different manufacturers.

CHAPTER FIVE

5.0 DISCUSSION

Contamination of weaning food may occur as a result of poor hygiene of the personnel, the equipments used and air quality of the environment where the weaning food preparation takes place (Badau *et al.*, 2005). The preliminary processes such as roasting at high temperature eliminate microorganisms and at the same time the presence of micro-organisms may be due to the sugar and other ingredients added (Badau *et al.*, 2005). The study has revealed that there are high levels of microbial contamination in the formulated complementary foods in the study area, especially for TPC and Moulds (Table 4 and 6) a phenomenon resembling to other studies conducted in many less developed countries, mostly African and Asian. The results for TPC were unsatisfactory and the same was reported from Northern Nigeria (Anigo *et al.*, 2010). Infants fed an improved complementary food improve their growth (Lartey *et al.*, 1999).

The results show considerable variations of *E. coli* ranging from 0 MPN/g to 71 ± 58 MPN/g. *E. coli* was not detected in only two brands ($F_{1\text{-}15}$ and $G_{1\text{-}15}$) and observed to be highest in brand $D_{1\text{-}15}$ as shown in Table 5. The majority of *Escherichia coli* serotypes are not pathogenic, however, they are used as indicator organisms as one of the coliforms (Forsythe and Hayes, 1998). However, presence of pathogenic *Escherichia coli* strains e.g. *Escherichia coli* 0157:H7 and *Escherichia coli* 026:H11 can be of serious concern since they cause severe illness resulting into death (Forsythe and Hayes, 1998). In this study, moulds were observed to have high counts ranging between (2.32 ± 0.60) $x10^3$cfu/g and (8.01 ± 1.10) $x10^4$cfu/g (Table 6) whereby some infant flour meals were shown to contain *Aspergillus* spp., *Penicillium* spp. and *Cladosporium* spp. Microscopic morphologies

observed were similar to the description given by Pitt and Hocking (1997). Presence of these fungi implies the possibility of the presence of toxin contaminants in the infant flour meals in the study area and thus need for more research becomes a critical issue. The problem is more serious in tropical countries of the world where humidity is high and the temperature is conducive for the growth and production of fungal toxins, e.g. Aflatoxins which are potent carcinogens, mutagens, teratogens, and immunosuppressants (Oluwafemi and Ibeh, 2011). The research results are similar to those observed by Oluwafemi and Ibeh (2011) in Nigeria on Microbial contamination of seven major weaning foods.

Similar situation was reported by Muhimbula and Issa-Zacharia (2010), that Tanzania being one of the developing countries is challenged with microbiologically contaminated complementary foods with pathogens exceeding the minimum acceptable limits. Another study that was done by Kung'u *et al.* (2009) in Zanzibar, revealed high level of bacterial contamination in formulated weaning foods commonly used in Zanzibar whereby the levels of aerobic bacteria, coliform and Enterobacteriaceae exceeded the acceptable levels for the bacterial quality of ready-to-eat foods. However, all sorghum based complementary foods require cooking before their consumption (Makinde and Ladipo, 2012) and therefore the same is true for other infant food ingredients.

5.1 Sample Composition and Level of Contamination

Table 3 shows different ingredients of the study samples and that all of them contained from 5 to 8 ingredients. The ingredients consisted of the following food items: rice, maize, soybean, ground nuts, finger millet, sorghum, wheat, vegetable, carrot, fish and pearl millet. Sample $A_{1\text{-}15}$, $D_{1\text{-}15}$, $F_{1\text{-}15}$ and $G_{1\text{-}15}$ had five ingredients, Sample $C_{1\text{-}15}$ had six ingredients, sample $B_{1\text{-}15}$ had seven ingredients and sample $E_{1\text{-}15}$ had eight ingredients. Maize and soybean were used in all seven samples, rice, groundnuts and finger millet were

used in six samples, sorghum was used in three samples, Wheat was used in two samples only while vegetable, carrot, fish and pearl millet were used in one sample each. Sample $D_{1\text{-}15}$ with five ingredients was observed to have highest counts of total microorganisms (TPC) and *E. coli* contaminants, whereby sample $E_{1\text{-}15}$ with highest number of ingredients was observed to have highest counts of fungal contaminants. Sample $B_{1\text{-}15}$ had lowest counts of TPC (Table 4), sample $F_{1\text{-}15}$ and $G_{1\text{-}15}$ did not have any *E. coli* (Table 5),sample $A_{1\text{-}15}$ having five ingredients had lowest counts of fungi contaminants (Table 6). By considering sample ingredients and fungal contaminants as per Table 3 and Table 6 respectively, it can be observed that there is a possibility of increase in fungal load simultaneously with increase in ingredient varieties. Although there are many sources of possible microbial contamination of cereals, it can be stated that all are traceable to the environment in which they are grown, handled, and processed (Bullerman and Bianchini, 2009) and because of this there is a need for entrepreneurs to efficiently follow the proper production guidelines so as to minimize contamination and attain the required quality of their cereal products.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The study has revealed that the microbiological quality of formulated infant flour meals available in the local shops in Morogoro Municipality is low. To some extent having high numbers of microorganisms in the food product is not an issue especially for those foods such as infant foods which must be heated before consumption, however importance comes when the particular food product contains pathogenic microorganisms which are heat stable and sometimes produce heat stable toxins. In this context it is important to take serious actions of preventing contamination even if the microorganism numbers are very low counts in the food products.

In this study, fungal pathogens such as *Aspergillus* spp., *Penicillium* spp. and *Cladosporium* spp. were observed in the FIFM studied. No further analyses were done to confirm the presence of pathogenic bacteria strains such as *E. coli* 0157:H7 due to limited time and resources. Since the indicator organisms (*E. coli*) were found, there is a high possibility for other pathogenic microorganisms to exist in the FIFM available in Morogoro shops and other areas in the country. Health effects of target fungal contaminants have already been defined in the literature review of this study.

Some of the samples analysed in this study contained addresses indicating that they were manufactured from other parts of Tanzania but away from the study area e.g. from Arusha Region, Iringa Region and Dar es Salaam Region. The issue of microbiological quality is crucial and needs to be looked into in order to prevent health problems that can result from

consumption of contaminated flour meals. Most of the formulated infant flour meals are consumed by infants at their weaning periods and thus both chemical and microbiological qualities should seriously be considered in order to have a healthy looks and healthy population. Exclusion of quality aspects of formulated infant flour meals and other food varieties will result into health problems.

It is concluded from this study that, the formulated infant flour meals from entrepreneurs are highly microbiologically contaminated in terms of TPC, *Escherichia coli* and moulds. This calls for more research to be conducted in order to have a wide understanding of the quality status of the products. There is a need for government institutions responsible for food safety to increase their efforts of food surveillance and inspection and initiate sustainable intervention measures to improve the microbiological qualities of formulated infant flour meals that will comply with all quality requirements in order to protect the consumer.

6.2 Recommendations

Basing on this study, the issue of microbiological quality of formulated infant flour meals will help consumers to be aware of the microbiological quality of infant foods. I recommend to the Government that institutions such as TBS, TFDA, TFNC and related academic institutions where the issue of quality in foods can be handled through scientific research should be supplied with adequate and important requirements for performing their duties.

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APPENDICES

Appendix 1: ANOVA table for TPC

Source of variation	Sum of squares	d.f	Mean square	*F* statistic	p-value[1]
Between brands	11.6473	6	1.94121	258.829	9.0×10^{-58}
Within brands	0.735	98	0.0075		
Total	12.3823	104			

Appendix 2: ANOVA table for *Escherichia coli*

Source of variation	Sum of squares	d.f	Mean square	*F* statistic	p-value[1]
Between brands	51.3026	6	8.55043	74.1949	3.12×10^{-34}
Within brands	11.2938	98	0.115243		
Total	62.5964	104			

Appendix 3: ANOVA table for Fungi

Source of variation	Sum of squares	d.f	Mean square	*F* statistic	p-value[1]
Between brands	27.8991	6	4.64989	795.819	9.1×10^{-81}
Within brands	0.5726	98	0.00584286		
Total	28.4717	104			

[1] p-value (two-tailed)