

**MICROBIAL QUALITY OF SPICES SOLD IN OPEN AIR MARKETS IN
LUSAKA DISTRICT**

BY

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**A RESEARCH REPORT SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF
MASTER OF SCIENCE IN ONE HEALTH FOOD SAFETY**

UNIVERSITY OF ZAMBIA

School of Veterinary Medicine

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DECLARATION

I, **JUDITH MUTAMBO**, do hereby declare that this research report represents my own work original work. It has been presented in accordance with the guidelines for the MSc research report of the University of Zambia. It has not previously been submitted for a degree, diploma or other qualification at this or any other University.

Signed: _____ Date _____

CERTIFICATE OF APPROVAL

The University of Zambia approves the research report submitted by JUDITH MUTAMBO, as fulfilling the partial requirements for the award of the Master of Science in One Health Food Safety by the University of Zambia.

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ABSTRACT

Spices refer to dried parts of plants used in food to enhance flavor, add color and make food more acceptable. As a result spices are widely used in the food industry where they may carry a large number of bacteria and mold that could contaminate the food. The objective of this study was to establish the presence and levels of microbial contamination in spices sold in open air markets in Lusaka District. The spice samples were collected randomly from various markets and subjected to bacterial quantification followed by culture, isolation and identification of bacteria and fungi.

Thirty nine (39) different types of spices were identified. Almost all the samples had a total bacteria plate count of colony forming units (cfu/g) above 1×10^5 . The total coliform count ranged between 1.0×10^2 and 5.0×10^6 cfu/g while the highest *E. coli* count was 3×10^4 cfu/g. For *Staphylococcus*, the range was between 1.0×10^2 and 1.5×10^6 cfu/g while some spices and spice blends had coliforms without detecting *E. coli*. The predominant bacteria were *Escherichia coli* and *Staphylococcus* species while the molds included *Mucor* genera and *Aspergillus* species. Some of the organisms identified like *Salmonella*, *Listeria*, *Staphylococcus*, *E. coli*, *Bacillus*, *Mucor* and *Aspergillus* are of concern as they may be implicated in life threatening invasive infections especially in immune compromised individuals.

This study determined that spices and spice blends have a high microbial load depicting a microbiological problem. Under favourable conditions, these microorganisms may become vegetative and multiply to infective and toxic levels thereby resulting in illnesses. It is therefore recommended that regular checks must be conducted for microbiological contaminants through surveillance so that control along the supply chain can be enhanced beginning from the import entry points.

DEDICATION

This study is dedicated to my late parents, my dear father Mr Dyson Mutambo (MHSREP) and Mother Mrs Selita Nakamba Mutambo (MHSREP), my husband Dereck Muyenga, my children Imanga, Sepiso, Lilato, and Tumelo for their sacrifice and encouragement throughout the study period.

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TABLE OF CONTENTS

	PAGE
Declaration.....	i
Certificate of Approval.....	ii
Abstract.....	iii
Dedication.....	iv
Acknowledgement.....	v
Table of contents.....	vi
List of Tables.....	ix
List of Figures.....	x
List of Abbreviations.....	xii
List of Appendices.....	xiii
CHAPTER ONE	1
INTRODUCTION.....	1
1.1 Background Information	1
1.2 Statement of the Problem	3
1.3 Justification	4
1.4 General Objective.....	4
1.5 Specific Objective	4
1.6 Research question.....	5
CHAPTER TWO	6
LITERATURE REVIEW	6
2.1 General overview	6
2.2 Forms of spices.....	7
2.3 Spice Production	7
2.4 Quality and Safety of Spices	8
2.4.1 Factors affecting Quality of Spices.....	8
2.4.2 Microbiological Standards for Spices.....	9
2.5 Imported and Locally Produced Spices.....	9

CHAPTER THREE	11
MATERIALS AND METHODS	11
3.1 Study Setting	11
3.2 Study Design	12
3.3 Sample Collection/Ethical consideration	12
3.4 Inclusion and Exclusion Criteria	12
3.5 Sample Size Determination	12
3.6 Sample analysis	13
3.6.1 Quantitative estimation of Bacterial population	13
3.6.2 Isolation,Culture and identification of Bacteria.....	13
3.6.3 Culture and Identification of Fungi.....	14
3.7 Data Analysis	14
CHAPTER FOUR.....	15
RESULTS	15
4.1 Spices sampled and identified in the study.....	15
4.2 Microbiological quality of spices and spice blends	17
4.3 Microorganisms identified	20
4.3.1 Bacterial organisms identified from spice and spice products.....	20
4.3.2 Fungi identified from spices and spice products	23
CHAPTER FIVE	24
DISCUSSION	24
5.1 Spice Utilization.....	24
5.2 Microbial quality of spices.....	24
CHAPTER SIX	28
CONCLUSION AND RECOMENDATIONS.....	28
6.1 Conclusion	28
6.2 Recommendations.....	28

REFERENCES.....	29
APPENDICES.....	37

LIST OF TABLES

	PAGE
Table 4.1: Profile of spices identified and sampled from the markets	16
Table 4.2: Microbiological analysis of spice and spice product samples	19

LIST OF FIGURES

	PAGE
Figure 1: Illustrations of commonly available spices	1
Figure 2: Map of Lusaka	11
Figure 4.1: Type of spices, spice blends and extracts sampled during the study.	15
Figure 4.2: Number of samples from each market	17
Figure 4.3: Plates indicating the colony forming units on plate count agar	20
Figure 4.4: Bacteria identified from the spices and spice blends	21
Figure 4.5: Frequency of morphological bacteria	21
Figure 4.6: <i>E. coli</i> as the predominant bacteria isolated from the various study areas	22
Figure 4.7: Distribution of <i>Staphylococcus</i> species in study areas	22
Figure 4.8: <i>Mucor</i> (a) and <i>Aspergillus</i> (b) identified from spices and spice blends	23
Figure 4.9: <i>Aspergillus</i> species identified from markets	23

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LIST OF ABBREVIATIONS

ASTA	World Health Organization
ESA	European Spice Association
FAO	Food and Agriculture Organization
FDA	Food and drug administration
CDC	Centers for Disease Control
cfu	Colony Forming Units
CSO	Central statistical Office
DAFF	Department of Agriculture, Forestry and Fisheries
ITC	International Trade Centre
HACCP	Hazard Analysis of Critical Control Points
NEDFI	North Eastern Development Finance
UNIDO	United Nations Industrial Organisation
SDA	Sabouraud Dextrose Agar
WHO	World Health Organization

LIST OF APPENDICES

	PAGE
Appendix 1 Bacteria identified from the spices and spice blends collected from the sampling markets.	37
Appendix 2 A summary of Bacterial organisms identified from each spice and spice product type collected from single markets	39
Appendix 3 A summary of Fungi identified from each spice and spice blends collected from single markets	42

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The word spice is primarily used to refer to aromatic vegetable substances which may sometimes be whole, broken or ground. They may be produced from root, bark, leaves, bulb, stems, flowers and seeds of certain plants. Spices contribute aroma, pungency, taste flavour and colour to food (Takeda *et al.*, 2008). Utilisation of spices is in different forms such as fresh, ground, whole and dried, as oil distillates or oleoresin extracted as solvents (UNIDO and FAO, 2005). A variety of spices are indicated in Figure 1 as an illustration.



Figure 1: Illustrations of commonly available spices (Anonymous accessed 2018; <https://thumbs.dreamstime.com/z/variability-asian-spices-wooden-table-traditional-oriental-indonesian-bowls-46649898.jpg>)

Spices have been important to mankind since the beginning of history. Several mythological evidence including “Epic of Gilgamesh,” and the “Bagavad Gita,” suggest their use for several purposes. Due to their strong preservative quality, spices such as myrrh, cumin, and cinnamon were used for embalming the dead (Bhagya *et al.*, 2017). According to Kuk, (2014), spices also help to maintain the balance of the body humors. Besides these, spices have been used to change the physical appearance of food. For instance, pepper and turmeric changed the color, appearance and the taste of food with many more health benefits. Ginger, nutmeg and cinnamon improve

digestion. They are also considered good for alleviating spleen diseases and sore throats (Prasad *et al.*, 2011).

Global spice production is dominated by Asia which is the largest spice producing continent in the world. Countries such as India, Indonesia, China, Vietnam, Guatemala and Bangladesh are top spice producing countries in the world (Takeda *et al.*, 2008). These spices are widely used in many countries as ingredients in food for enhancement of flavor, taste and for aesthetic value. Some spices may be common in a region because the climatic conditions such as temperature and humidity favour their growth (Bakobie *et al.*, 2017). The use of these spices in such areas become embedded in people's cultures and way of life as they form part of their traditional practices (ITC, 2015). Human migration has led to spice use all over the world, as peoples cultures are transferred to new areas of habitation. This has led to spice use everywhere in the world. Africa has had a lot of migrants who have settled. In most of African cultures there is abundant use of herbs and spices as they are believed to have an impact on human health (Egharevba and Gamaniel, 2017).

The spice industry in Zambia is still in its infancy, although there has been considerable growth in spice imports from the year 2010 to date, indicating a rise in demand (Fdii, 2013). This could be attributed to the influx of foreign expatriates that may have caused changes in the food consumption patterns, and the change in lifestyles of individuals given their new appreciation of benefits linked to spices and an increase in income. Imported consumer packed spices that meet international standards are expensive as compared to the locally produced spices.

In Zambia, production of spices has seen considerable investment. Commercialization of paprika and chili production in the Northern Province of Zambia has received significant investment (Fdii, 2013). The popularity of spices on the Zambian market has been driven by high consumer demand for these products for use in food preparation, medicinal and cosmetic purposes. This is because they have been widely marketed for body wellness and preventive medicine (Bhagya *et al.*, 2017). Such spices include ginger, garlic, cumin, rosemary, cinnamon and turmeric.

The retail spice trade in Zambia is dominated by dried spices. Most spices on the Zambian market are imported in their dried form from Asia, South Africa, and other African countries in bulk

(DAFF, 2007). They are delivered to Lusaka city and stored in warehouses and storage sheds where they are sold on wholesale to retail traders and other food establishments. Spices are generally repacked into small retail packs ranging from 500 grams to 1 kilogram (Kg) packs. Packaging involves the use of polythene bags, and Phenol or PET containers. The packaging is done manually from anywhere the trader may choose. Some traders package their spices from their retail outlets while others package from their homes. This scenario may result into microbial contamination of these spices as observed by other workers (Garbowska *et al.*, 2015). The resulting microbial contamination that may result during packaging may be overcome during food preparation as cooking can inactivate bacteria.

In view of the above, this study was aimed at determining the microbiological quality of spices sold on retail in open air markets. The relevance of this study was to fill the knowledge gap regarding the presence of microbial contaminants and the levels of microbial contamination in spices offered for sale in Lusaka District, particularly in the open air markets.

1.2 Statement of the Problem

Circumstantial evidence has shown that spices are not subjected to microbial investigation prior to entry into the country. Although spices are generally used for their aesthetic properties, spices and herbs can often be a major source of microbial contamination. In some studies, fungi and bacterial of significance has been documented to have been isolated from spices (Ahene *et al.*, 2011).

Spice trading is an economically viable business due to the value attached to the use of these products (FAO, 2011; Terzi *et al.*, 2010). In Zambia most of the spices found on the market are imported with an exception of paprika and chilies that are produced in Northern Province of Zambia (Fdii, 2013). Imported spices originate from Asia, Europe and other regions via South Africa and from other African countries such as Ethiopia, Tanzania and Ghana. The spices originating from some of the exporting countries were found to contain microbial contaminants according to studies conducted in Ghana by Bakobie *et al.*, (2017) and Sri Lanka, India and Bangladesh by Takeda *et al.*, (2008). Spice trade has become common in Lusaka district and a need was realized to check the microbial quality of these spices.

1.3 Justification

This study was timely in the sense that the researcher could not find published data or evidence regarding the microbial quality of spices found on the Zambian market. Spice imports in Zambia have increased over the years (Fdii, 2013). It has been established that spices are not subjected to routine microbial investigations at ports of entry prior to clearance and certification by the authorities (Food and Drugs Administration, 2013) despite evidence being available that spices could be contaminated with bacteria (Bakobie *et al.* 2017). Some of these bacteria could be very significant in food safety. Furthermore, the production systems may subject the spices to bacterial contamination from soil before harvesting hence the need to screen spices for contaminants. Ahene *et al.*, (2011) investigated fungal and bacterial contamination of spices and showed that spices contain various types of microorganisms such as pathogenic bacteria and toxigenic molds.

It was anticipated that this study would generate knowledge that would highlight the safety of the spices being sold on the Zambian market. The study would further raise awareness among consumers regarding the possible microbial contaminants in spices. This study would also be a milestone for food safety in Zambia as no information regarding the microbial quality and safety of spices that are locally produced or imported in Zambia is available. This study would henceforth be a basis for further scientific enquiries into the risks associated with use of contaminated spices through characterization of microbial contaminants of imported and locally produced dry spices sold in local markets.

1.4 General Objective

The objective of the study was to determine the microbial quality of spices sold in open air markets in Lusaka district, Zambia.

1.5 Specific Objectives

1. Establish the presence of microbial contaminants in spices sold in open air markets in Lusaka district.
2. Establish the levels of microbial contaminants in spices sold in open air markets in Lusaka district.
3. Isolate microorganisms from spices sold in open air markets in Lusaka district.

1.6 Research Questions

1. Do spices have microbial contaminants?
2. What are the levels of microbial contamination?
3. What species of bacteria and fungi can be isolated from spices?

CHAPTER TWO

LITERATURE REVIEW

2.1 General overview

Since ancient times, spices have been used for culinary, medicinal, cosmetic, and spiritual purposes. Many traditional foods around the world are cooked with spices to enhance their flavor, color, and to improve their keeping quality. Spices are derived from any part of a plant that is not a leaf, such as the root, stem, bulb, bark or seeds. More than 100 varieties of spices are produced throughout the world (Gottardi *et al.*, 2016). Asia is the main leader for the production of spices, particularly of cinnamon, pepper, nutmeg, cloves, and ginger, while Europe grows mainly basil, bay leaves, celery leaves, chives, coriander, dill tips, thyme, and watercress. In America, instead, pepper, nutmeg, ginger, allspice, and sesame seed are mainly produced (Prasad *et al.*, 2011). Although spices have been used (mostly dried seed, fruit, root, bark, or vegetative material) for rituals, cosmetics and perfumery, their flavoring, coloring and, especially, preservative properties have been widely applied both in the traditional food preparations and in the conventional food industry (Egharevba and Gamaniel, 2017; Meharwade *et al.*, 2017; Vitullo *et al.*, 2011). In fact, many compounds isolated from spices have shown antimicrobial activity against some of the most common microorganisms that affect the food quality and shelf life (Tajkarimi *et al.*, 2010).

Some natural spices have been identified to exhibit antioxidant properties due to their chemical compounds especially to phenolic compounds, in fact there is a linear relationship between the phenolic content and the antioxidant activity of a spice. Essential oils, oleoresin and other spice extracts contain important antioxidant activity which can be profited by food industry (Wojdyło *et al.*, 2007). There are several studies that consider antioxidants as defense mechanisms in the body against cardiovascular diseases, cancer, arthritis, asthma and diabetes. This has led to increased use of natural sources of antioxidants (Peter and Shylaja, 2012).

The introduction of spices through the meals has various beneficial effects as well. For instance, they can stimulate the secretion of saliva, promote digestion, prevention from cold and influenza, reduction of nausea and vomiting (Sultana *et al.*, 2010). The microbial quality of spices is mainly

dependent on the handling practices implored during harvesting, processing, transportation and storage of these products prior to use in food preparation (Bakobie *et al.*, 2017). Other sources of contamination could be environmental conditions prevailing especially in open air markets (UNIDO, 2017; Garbowska *et al.*, 2015). Spices produced in India, Indonesia, Bangladesh, Sri Lanka and Ghana have been assessed for quality in a number of studies where the presence of microbial contaminants was established. The presence of these contaminants was attributed to handling practices during harvesting, processing, storage and transportation conditions (Takeda *et al.*, 2008).

2.2 Forms of spices

Generally, spices are sold in various forms. These include whole spices (cardamom, black pepper, clove, turmeric, ginger, cinnamon, cassia), seed spices (celery, fennel, cumin, fenugreek), powdered or ground spices (turmeric, chilies, ginger), spice mixes (curry powders and masalas), paste (curry paste, vindaloo paste, ginger-garlic paste), concentrates (tamarind concentrate), oils and oleoresins (Meharwade *et al.*, 2016).

2.3 Spice Production

International production of spices is dominated by South Asia (India, Pakistan and Nepal) which is by far the world's largest spice producing region and produces more than 55 percent of spices worldwide. East Asia (China) and South-East Asia (Indonesia, Vietnam and Thailand) are the second largest spice producing regions in the world. India is the single largest producer, consumer and exporter of spices in the world (Lamba *et al.*, 2015). The country produces about 75 out of 109 varieties listed by international organization for standardization ISO and accounts for half of the global trading in spices (Herms, 2015)

For centuries, Africa has been an important player in the global spice trade. Currently, the African continent produces less than 12 percent of the spices produced worldwide (Herms, 2015). According to the Food and Agricultural Organisation (2011), some of the leading producers of spices in Africa include Nigeria, Madagascar, Tanzania, Uganda, Kenya and Ghana. Although the soil and climate in many parts of Africa are favourable to growing spices, the huge opportunities in these widely traded commodities have not been fully exploited (UNIDO, 2017; FAO, 2011).

The scale of commercial production of spices in Zambia is undocumented although production of paprika and chili pepper in the Northern Province of Zambia has seen considerable investment. This sets a platform for tangible potential for growth in the sector (Fdii, 2013). Spice trading has also become a source of livelihood for people living in many third world countries such as Mexico, Nepal, Thailand, Ethiopia and Zimbabwe, (FAO, 2011). Some spices are sold in their natural state while others are sold as blends of different spices in order to provide variety and enhanced flavor. Some spices such as birds eye chilies and ginger have been known to originate from within African countries like Malawi, Rwanda and Ethiopia (Herms, 2015). Nigeria has also been reported to be producing spices with pharmaceutical prospects (Egharevba and Gamaniel, 2017).

2.4 Quality and Safety of Spices

Production of spices is higher in non-industrialized countries with little or no food production safety control and minimal technology for hygienic processing (Vitullo *et al.*, 2011). The American spice trade association guided that processors in exporting countries may not often subject spices to treatment (ASTA, 2017). This concern demonstrated the potential for contamination of spices during the producer consumer continuum (Sagoo *et al.*, 2009). Studies have linked *Salmonella* and other significant microorganisms have been associated to food borne disease outbreaks (Dennis *et al.*, 2013; FDA, 2013).

2.4.1 Factors affecting Quality of Spices

Spices are affected by the moisture content, specifically spices in powder form, are hygroscopic in nature and pick-up moisture from the atmosphere resulting in sogginess and caking/lumping of the powder. Pick-up of moisture also results in loss of free-flowing nature of the spice powder (Pejic *et al.*, 2015).

Loss of aroma/flavour is common in spices that contain volatile oils such as sesame seeds oregano and rosemary (Figiel *et al.*, 2010). These oils impart the characteristic aroma/flavour to the product. Loss in the volatile oil content or oxidation of some aromatic compounds result in loss of aroma and flavor (Singh, 2013).

Discolouration is common in spices such as green cardamom, red chilies, turmeric and saffron contain natural pigments (Elizabeth *et al.*, 2015). This is due to light affecting the pigments resulting in fading or colour deterioration.

Insect infestation in spices is common if pre and post-harvest conditions were poor. Spices may be prone to spoilage due to insect infestation, which can be further accelerated due to high humidity, heat and oxygen (ITDG, 2013).

Microbial contamination is common in high humidity conditions of 65% and above, moisture absorption occurs (Garbowska *et al.*, 2015). Beyond a certain level of moisture content, spoilage due to microbial growth sets in (Pejic *et al.*, 2015).

2.4.2 Microbiological Standards for Spices

Microbial quality in spices can be determined by the laboratory detection of microbial contaminants in spices. The analysis of contaminants in spices and the guiding standards are illustrated in the Codex Code of Hygienic Practice (Codex, 1995). The codex specifies that dried spices and herbs should be free from pathogenic microorganisms at levels that may pose potential danger to health and further requires that *Salmonella* should be absent in treated ready-to-eat spices. The European Spice Association also specifies that *Salmonella* should be absent in 25g of spice, *Escherichia coli* to be present at less than 10^2 cfu/g (Yankey, 2014; European Spice Association, 2011).

2.5 Imported and Locally Produced Spices

A wide range of spices in Zambia are imported. The imported spices find their way into the country through the international ports of South Africa and Tanzania (DAFF, 2017). The imported stock includes: pepper (king of spices), cardamom (queen of spices), chilies, ginger, turmeric, coriander, cumin, vanilla, rosemary, garlic and clove. Major spice source countries are developing countries whose food safety systems are still developing (ASTA, 2017). This means that improvements in the food safety controls can significantly improve the quality of spices consumed in Zambia. India has employed a strategy that involves creation of spice parks where producers and aggregators

may take spice to a central place to be cleaned, treated and tested before supply (Dennis *et al.*, 2013).

Locally produced spices that are commercialized include chili peppers and paprika. Chili pepper is mostly used as a flavor enhancer in food preparation while paprika is a spice mainly used in food production for both flavor and aesthetic reasons. Chili pepper and paprika are mostly sold on the local traditional markets or open air markets. Circumstantial evidence shows that the product is subjected to some form of processing before being packaged in bulk and offered for sale (FAO, 2014). Locally produced spices are mostly unprocessed at point of sale. These spices are sold in open air markets in sacks.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Setting

The study was conducted in Lusaka District, Lusaka province, which is located on the southern part of the central plateau at an elevation of 1300 meters. Lusaka is the capital city of Zambia as well as the centre for both commerce and government (Central Statistics Office (CSO), 2011). Lusaka District has the total population of 1, 747, 152 (CSO, 2012), and population density of 4853.2 persons per square kilometre (CSO, 2010). The location of the study area was appropriate and convenient for ease of sample collection. The samples were drawn from 4 randomly selected sub districts that included the Central business district, Chilenje, Chawama and Chelstone.

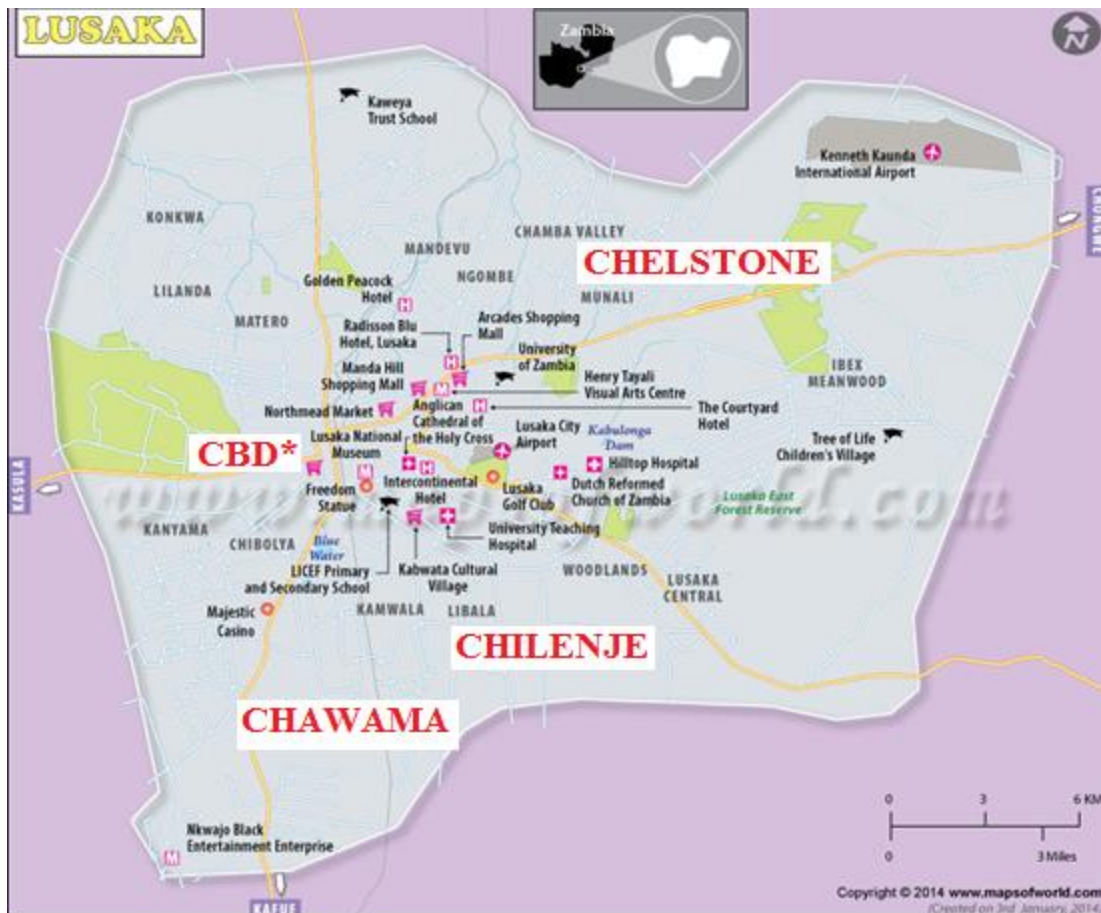


Fig 2: Map of Lusaka (www.mapsofworld.com), showing the study area Chawama sub district, Chilenje sub district, Chelstone sub district and the CBD*(Central Business District).

3.2 Study Design

A quantitative approach was adapted in this study with the use of an experimental design to isolate and identify the species of microbial contaminants through a collection of spice samples offered for sale in open air markets. Spice samples from the trading points were the subjects of the study. There was no manipulation of the any independent variable in this study.

3.3 Sample Collection/Ethical consideration

Purposive sampling was used in this research due to limited number of spice dealers. All stands with spices were targeted for sampling in Soweto Market (Central Business District), Mutendere (Chelstone sub district), Chawama market (Chawama sub district) and Libala Tuesday market (Chilenje sub district). At every point, approximately 100g of the identified spice type was sampled using aseptic techniques and placed in Zip lock bags and stored in cool boxes loaded with ice packs in order to maintain the microbial integrity. The spice products identified were then immediately transported to the laboratory for analysis. During sample collection, the spices were bought from the vendors just like any other customer buying for personal use. There was no interaction with the vendors. This study was aimed at identifying the microbial organisms found in spices.

3.4 Inclusion and Exclusion Criteria

All spices offered for sale in the open air markets were included in this study whereas spices not traded in retail shops outside the open air markets were not included in the study.

3.5 Sample Size Determination

Data on the proportion (p) of microbial contaminants in spices is unknown. The sample size for this study was based on Cochran's sample size formula (Cochran, 1977). Therefore, assuming we require the estimate to be within 5% of the true value in either direction, using the CI of 95%. , p = 0.5 to yield the maximum value. The sample calculation is based on the formula; $n = \frac{Z^2.pq}{d^2}$

Where:

Z = value of the standard normal distribution corresponding to a significance level of a (1.96 for $\alpha= 0.05$).

p = 0.5

$$q = 1 - p$$

$$d = 0.05.$$

This formula gives a sample frame of 384 samples. The corrected finite population formula was applied as follows;

$$n = \frac{1}{\frac{1}{n} + \frac{1}{n}} = n = \frac{1}{\frac{1}{384} + \frac{1}{57}} = n = \frac{1}{0.02014803} = 49.632644$$

49. 632644 or 50 samples in each sub district to be involved in the study.

This study was limited with time therefore only one (1) market in each Sub district was targeted for sampling. Sampling was done in such a way that the spice varieties and samples collected were replicated depending on availability.

3.6 Sample analysis

3.6.1 Quantitative estimation of Bacterial population

The initial Bacterial flora in the spices was determined by transferring 25 g samples into 250 ml Erlenmeyer flasks containing 225 ml of 0.1% peptone water as a diluent. Each flask was shaken at 140 *xg* for 20 minutes on an orbital shaker. Serial dilutions up to 1:10⁴ were made and 100 μ l aliquots were plated on Brain Heart Infusion agar (Himedia, Mumbai, India) and Plate count agar (Himedia, Mumbai, India). All the plates were incubated at 37°C overnight. Colonies of Bacteria that appeared after incubation were counted and calculated as cfu/g sample as described by Ahene *et al.*, (2011) with minor modifications.

3.6.2 Isolation, Culture and identification of Bacteria

The suspension of the spices from section 3.6.1 was used for bacteria isolation. Briefly 1 ml of the spice suspension was placed in 9 ml of brain heart infusion broth (Himedia, Mumbai, India) and incubated overnight at 37°C. After overnight incubation, the broth was inoculated on nutrient agar (Himedia, Mumbai, India), blood agar (Himedia, Mumbai, India) and MacConkey agar (Himedia, Mumbai, India). These were then incubated overnight at 37°C for 24 hours.

The bacteria was identified by gram staining and biochemical reactions as described by Bisen *et al.*, (2012). Biochemical test identification of the isolated bacteria was carried out by first

observing the appearance of the colonies produced which was followed by Gram's staining and subsequent subculture onto Nutrient agar. The most commonly used techniques were Vitek 2 Compact and Analytical Profile Index (API) 20 E for rapid identification of microorganisms as previously described by Buszewski *et al.*, (2017).

3.6.3 Culture and Identification of Fungi

To culture fungi, a loopful of spices were placed on Sabouraud Dextrose Agar (HI media, Mumbai, India) and then incubated at room temperature for five days. The fungal growth on Sabouraud Dextrose Agar (SDA) was transferred to sterilized plates for purification and identification. The grown fungi were mounted on a slide, stained with lactophenol cotton blue covered with a cover slip and then examined under the microscope as previously described (Carter and Chengappa, 1991). Fungi was identified on the basis of their colony morphology and spore characteristics as well as critical examination of fungal structures based on cultural and morphological characteristics that included colony colour, size of sclerotia, texture and conidial morphology characteristics (Larone, 2011; Guarro *et al.*, 1999).

3.7 Data Analysis

The data was analysed by enumeration of the mean viable plate count, isolation and identification of pathogenic bacteria. Conclusion was drawn from reference to and comparisons with quality standards available locally and to applicable international standards. The data collected was entered into Microsoft excel and thereafter, exported to SPSS for processing and analysis. Presentation of results for this study is in form of histograms, charts, graphs and tables.

CHAPTER FOUR

RESULTS

4.1 Spices sampled and identified in the study

A number of spices and spice blends were sampled from open air markets and identified in this study. A total of 98 spice samples were collected involving 39 types as shown in Table 4.1. In this study spice blends were considered as mixtures of naturally occurring spices and herbs blended by the vendors.

Of the 39 samples collected, 55 percent were naturally occurring spices while 42 percent were spice blends given different trade names by the vendors. Only 3 percent were extracts from naturally occurring proteins as shown in Figure 4.1 below.

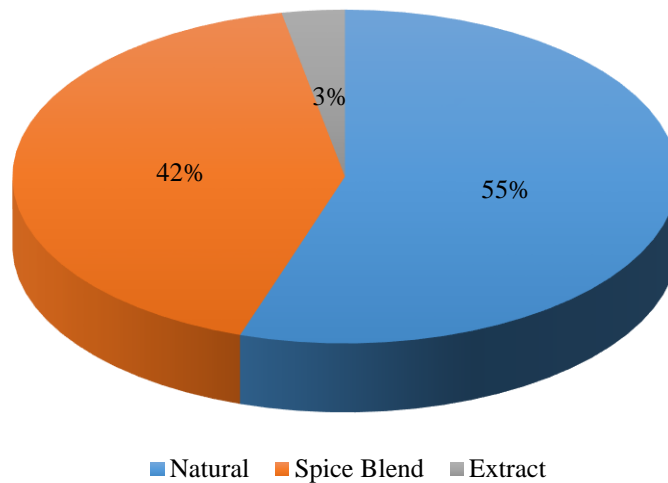


Figure 4.1: Type of spices, spice blends and extracts sampled during the study.

Table 4.1: Profile of spices identified and sampled from the markets

S N	Name of spice	Scientific Name
1.	Black Pepper	<i>Piper nigrum</i>
2.	Paprika	<i>Capsicum annum L</i>
3.	Turmeric	<i>Curcuma longa</i>
4.	Garlic	<i>Allium sativum</i>
5.	Cinnamon	<i>Cinnammomum verum</i>
6.	Black/White Pepper	<i>Piper nigrum</i>
7.	Chilli	<i>Capsicum annuum</i>
8.	Ginger	<i>Zingiber officinale</i>
9.	Chicken	Blend*
10.	Ranger Biltong	Blend
11.	Curry Powder	<i>Murraya koenigi</i>
12.	Coriander	<i>Coriandrum sativum</i>
13.	Fish Seasoning	Blend
14.	Samosa	Blend
15.	T Bone	Blend
16.	Shawama	Blend
17.	Beef Sausage	Blend
18.	Brown Onion Soup Powder	<i>Allium cepa L.</i>
19.	BBQ	Blend
20.	Chip Sprinkle	Blend
21.	Gravy	Extract**
22.	Beef Stew	Blend
23.	Spice For Rice	Blend
24.	Steak And Chops	Blend
25.	Amchur Powder	<i>Magnifera indica</i>
26.	Sāmbhar Masala	Blend
27.	Aromat	Blend
28.	White Pepper	<i>Piper nigrum</i>
29.	Paneer Tika	Blend
30.	Kitchen King	Blend
31.	Biryani Masala	Blend
32.	Garam Masala	Blend
33.	Cumin Powder	<i>Cuminum cyminum</i>
34.	Fenugreek Powder	<i>Triagonella foenumgraecum</i>
35.	Aarchur Masala	Blend
36.	Mustard Seed Powder	<i>Brassica hirta</i>
37.	Cayenne Powder	<i>Capsicum annum 'acuminatum'</i>
38.	Mix Lemon	Blend
39.	Clove	<i>Syzygium aromaticum</i>

Blend*; Mixture of different spices and herbs prepared by the vendors. Extract**; Fat and juice extracts from animal protein sources.

The samples collected were obtained from Libala Tuesday market in Chilenje sub district (40 percent), Soweto market from the Central Business district (33 percent), Mutendere market from Chelstone sub district (19 percent) and Chawama market from Chawama sub district (8 percent) as shown in figure 4.2. Chili pepper, paprika and turmeric were the most used spices as encountered from the number of vendors selling them and thus samples of these spices were available and collected from all the markets.

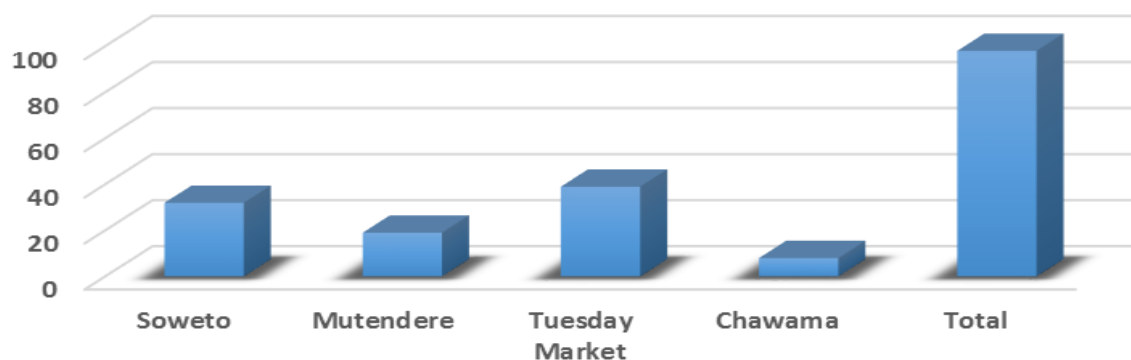


Figure 4.2: Number of samples collected from each market.

4.2 Microbiological quality of spices and spice blends

The spices and spice blends sampled were analysed for microbiological quality by conducting colony counts as shown in Figure 4.3. Almost all the samples had a total bacteria plate count of Colony forming units (cfu/g) above 1×10^5 with the maximum being 1.9×10^9 from Chawama market (Table 4.2). The total coliform count ranged between 1.0×10^2 and 5.0×10^6 cfu/g, while the highest *E. coli* count was 3×10^4 cfu/g. In some spices we could not record any count. In case of *Staphylococcus* the range was between 1.0×10^2 and 1.5×10^6 cfu/g. Interestingly some spices and spice blends had coliforms while no counts were recorded with *E. coli*.

Table 4.2: Microbiological analysis of spice and spice product samples

Sample Name	Total Plate count (cfu/g)	Total Colifom count	<i>Staphylococcus</i> count (cfu/g)	<i>E. coli</i> count (cfu/g)
Black Pepper	1.4X10 ⁷	5 X 10 ⁶	4 X 10 ⁴	2 X 10 ³
Paprika	6.0X10 ⁶	3.0 x 10 ⁴	2 X 10 ⁵	3 X 10 ²
Turmeric	4.8X10 ⁵	2.0 x 10 ⁵	0	0
Garlic	5.2X10 ⁶	3.0 x 10 ⁵	0	0
Cinnamon	2.1X10 ⁴	2.3 x 10 ³	2 X 10 ²	1 X 10 ²
Black/White Pepper	2.7X10 ⁵	6.0 x 10 ³	1 X 10 ³	0
Chilli Pepper	1.78X10 ⁶	1.2. x 10 ⁶	2 X 10 ²	1 X 10 ⁴
Ginger	6.16 X10 ⁶	14.0 x 10 ³	1.5 x 10 ⁶	0
Chicken	3.0X10 ⁵	2.0 x 10 ⁶	1.0 x 10 ²	0
Chilli Pepper	2.46X10 ⁵	7.0 x 10 ⁴	1.0 x 10 ³	2 X 10 ³
Tumeric	7.6X10 ⁷	5.0 x 10 ⁶	0	2 X 10 ⁴
Ranger Biltong	3.05X10 ⁶	1.0 x 10 ⁴	3.0 x 10 ⁴	1 X 10 ²
Curry Powder	7.04X10 ⁵	1.0 x 10 ⁵	2.0 x 10 ²	2 X 10 ³
Paprika	4.8X10 ⁵	2.0 x 10 ⁵	1.0 x 10 ³	1 X 10 ⁴
Tumeric	7.6X10 ⁵	3.0 x 10 ⁴	6.0 x 10 ²	2 X 10 ²
Chilli Pepper	4.64X10 ⁵	4.0 x 10 ⁴	3.0 x 10 ³	2 X 10 ²
Tumeric	6	1.0 x 10 ²	20	50
Corriander	4.04X10 ⁶	1.5 x 10 ⁶	0	0
Fish Seasoning	7.0X10 ⁵	1.4 x 10 ⁵	4.0 x 10 ²	2 X 10 ³
Rajah Curry	4.12X10 ⁵	0	0	1.5 X 10 ²
Samoosa	1.67X10 ⁶	2.0 x 10 ³	4.0 x 10 ⁴	0
T Bone	5.24X10 ⁵	1.0 x 10 ⁴	2.0 x 10 ²	3 X 10 ⁴
Shawama	4.44X10 ⁴	3.0 x 10 ⁴	3.0 x 10 ²	1 X 10 ²
Beef Sausage	2.12X10 ⁶	2.0 x 10 ³	0	0
Paprika	5.19X10 ⁶	3.0 x 10 ⁵	0	2 X 10 ⁴
Chili Pepper	1.75X10 ⁶	2.0 x 10 ³	0	0

Table 4.2: Microbiological analysis of spice and spice product samples continued:

Sample Name	Total Plate count (cfu/g)	Total Colifom count	<i>Staphylococcus</i> count (cfu/g)	<i>E. coli</i> count (cfu/g)
Brown Onion Soup Powder	1.55X10 ⁵	1.0 x 10 ²	2.0 x 10 ²	0
BBQ	2.68X10 ⁵	1.0 x 10 ²	0	0
Chip Sprinkle	1.43X10 ⁵	0	0	0
Gravy	1.16X10 ⁶	3.0 x 10 ³	1.0 x 10 ³	1 X 10 ³
Beef Stew	9.52X10 ⁴	2.0 x 10 ²	2.0 x 10 ⁴	0
Spice For Rice	6.72X10 ⁵	1.0 x 10 ²	1.0 x 10 ²	0
Steak And Chops	4.92X10 ⁵	2.0 x 10 ³	0	0
Amchur Powder	7.32X10 ⁵	2.0 x 10 ²	0	0
Sambhar Masala	7.72X10 ⁵	1.0 x 10 ²	0	0
Aromat	4.04X10 ⁵	2.0 x 10 ²	0	0
White Pepper	2.97X10 ⁵	3.0 x 10 ²	0	0
Paneer Tika	1.5X10 ⁷	1.5 x 10 ²	0	0
Kitchen King	4.4X10 ⁵	2.0 x 10 ²	0	0
Biryani Masala	5.55X10 ⁶	1.0 x 10 ²	0	0
T. Bone	5.64X10 ⁵	1.0 x 10 ³	4.0 x 10 ³	3.0 x 10 ²
Garam Masala	4.96X10 ⁵	2.0 x 10 ²	0	0
Cumin Powder	1.82X10 ⁵	1.0 x 10	0	0
Fenugreek Powder	4.93X10 ⁵	3.0 x 10	1.0 x 10 ³	0
Aarchur Masala	4.74X10 ⁵	1.0 x 10 ²	0	0
Mustard Seed Powder	3.98X10 ⁵	3.0 x 10 ²	0	1 X 10 ²
Cayenne Powder	1.49X10 ⁶	3.0 x 10	0	0
Mix Lemon	1.22X10 ⁶	1.0 x 10 ²	0	0
Clove	1.5X10 ⁶	4.0 x 10	0	0



Figure 4.3: Plates indicating the colony forming units on plate count agar.

4.3 Microorganisms identified

The spices and spice blends were found with a number of different species of microorganisms that included bacteria and fungi in all the samples collected and analysed.

4.3.1 Bacterial organisms identified from spice and spice products

The bacteria organisms identified are indicated in Figure 4.4. The dominant bacteria group to be isolated was Gram negative rods, with *E. coli* being the leading bacteria Figure 4.5. The predominant Gram positive was *Bacillus*. *Escherichia coli* was isolated in 54 percent of the samples analysed while *Staphylococcus* spp was identified in 55 percent of the spices analysed. *Escherichia coli* and *Staphylococcus* spp were the most predominant ones. The *Staphylococcus* spp identified was segregated into *Staphylococcus aureus* (63%) and the unidentified *Staphylococcus* species (37%). Appendix one and two outlines the samples and bacteria isolated and identified from each study area. Mutendere market recorded a higher number of contaminated samples. A summary of all bacteria identified from each type of spice or spice blends analysed are indicated in Appendix one. *E coli* was detected in samples collected from all the markets, with 20 isolates being identified from samples collected from Soweto market, 15 isolates from Mutendere market and 4 isolates from Chawama market (Figure 4.6). In case of *Staphylococcus*, the market distribution is shown in Figure 4.7.

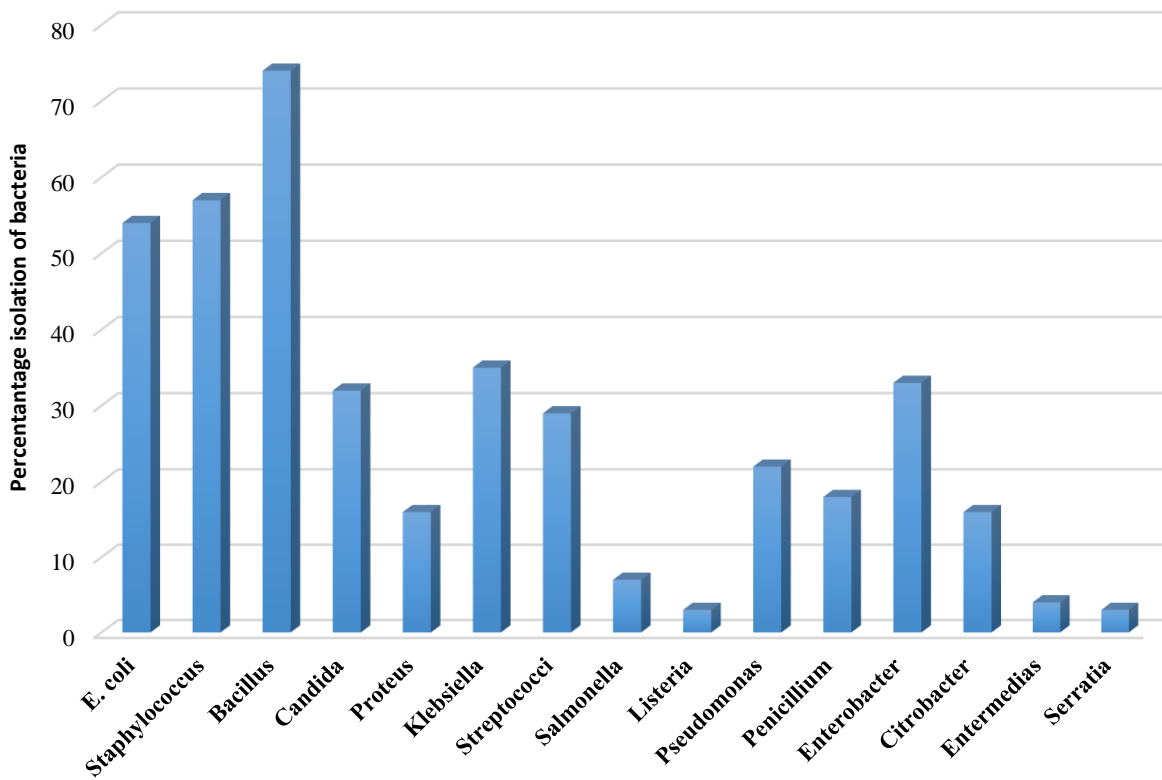


Figure 4.4: Bacteria identified from the spices and spice blends

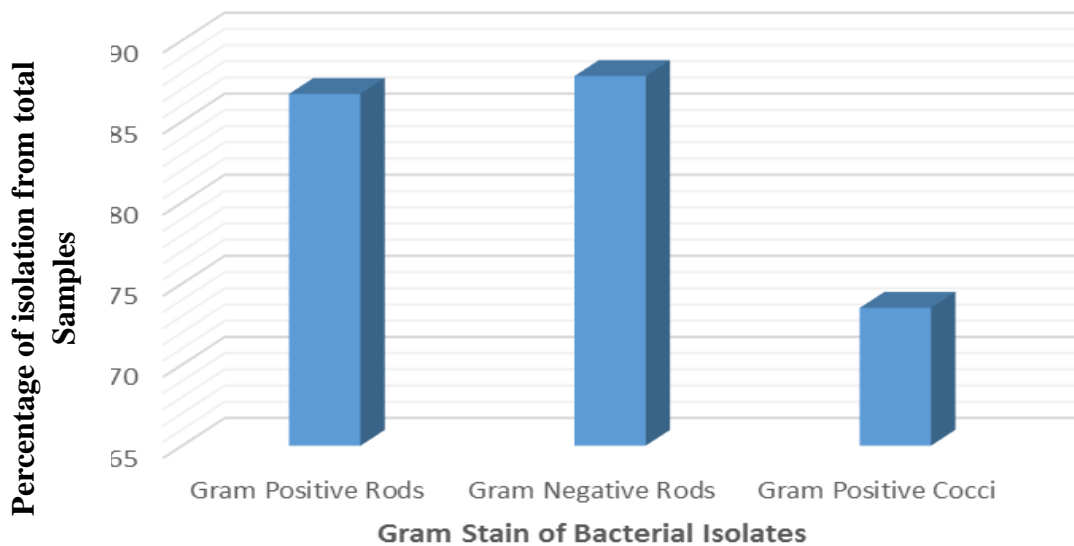


Figure 4.5: Frequency of morphological bacteria

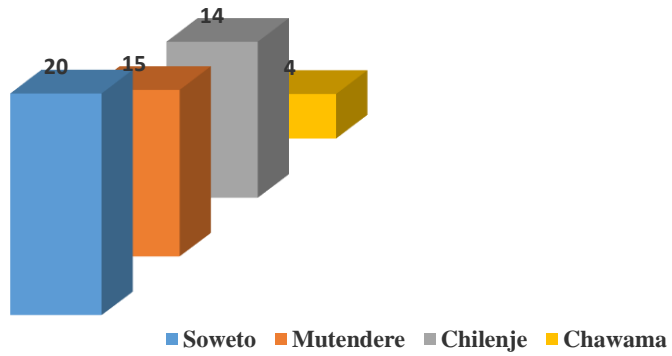


Figure 4.6: *E. coli* as the predominant bacteria isolated from the various study areas.

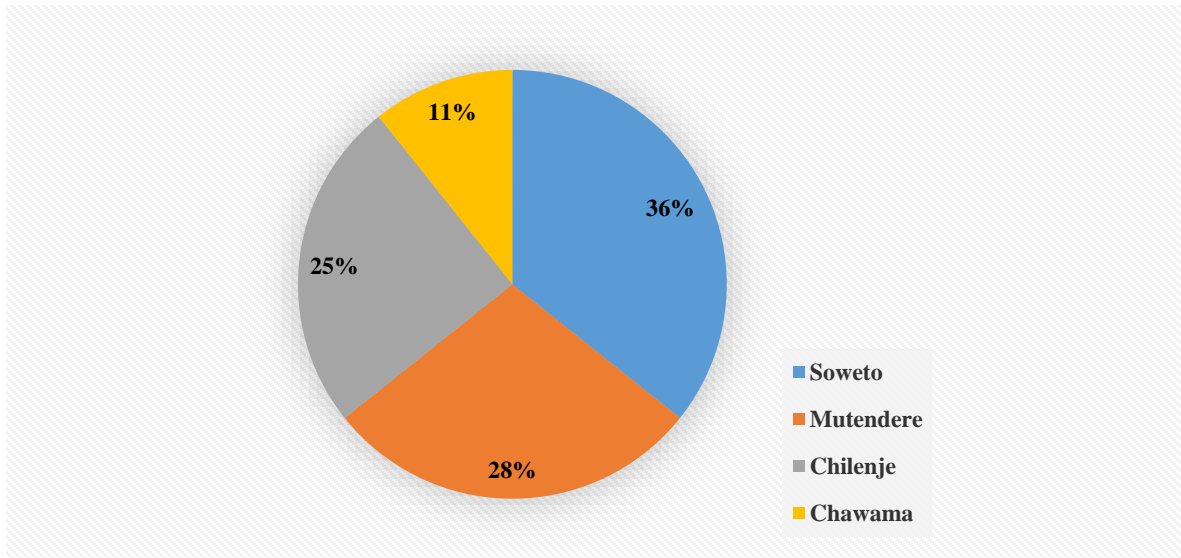


Figure 4.7: Distribution of *Staphylococcus* species in study areas.

Of significance is *Salmonella* and *Listeria* which were detected in the spices. *Salmonella* was detected in turmeric, paprika, steak and chops, amchur powder and chip sprinkle, whereas *Listeria* was detected in coriander only.

4.3.2 Fungi identified from spices and spice products

Some fungi genera was not isolated in some spices and spice blends. *Aspergillus* spp was found from all the markets under study as shown in figure 4.8, despite the fungi not being present in all samples collected. The distribution is indicated in Figure 4.9.

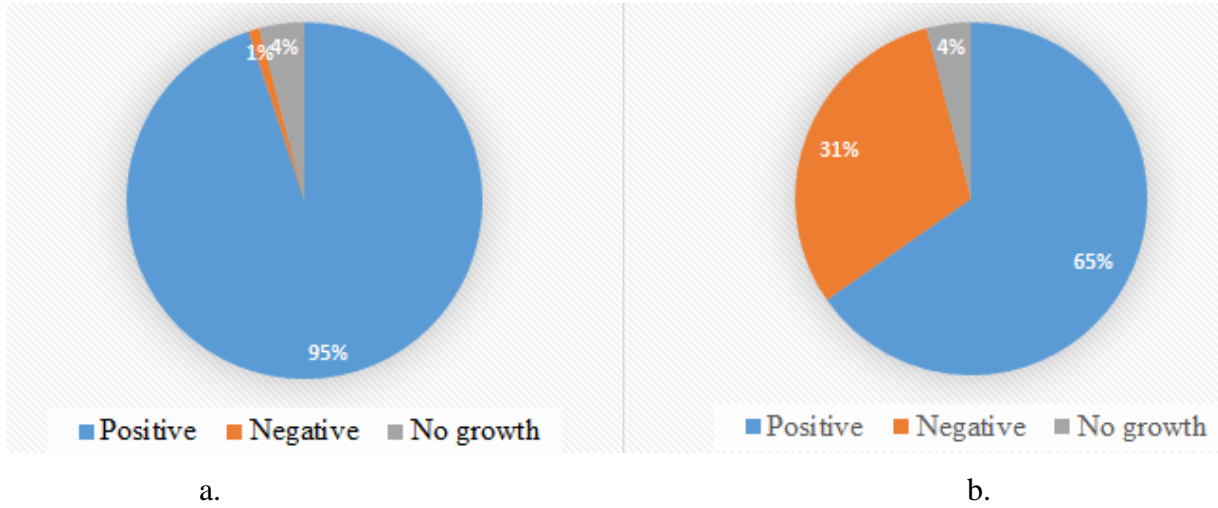


Figure 4.8: *Mucor* (a) and *Aspergillus* (b) identified from spices and spice blends

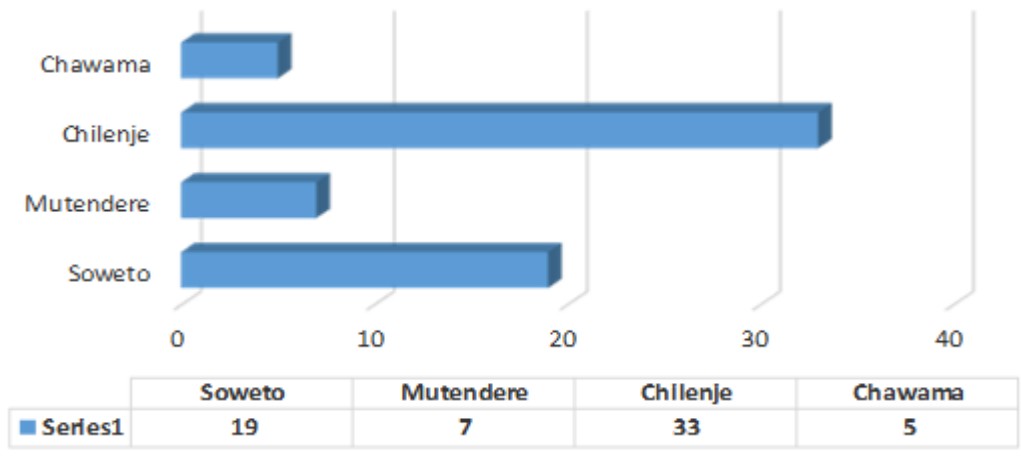


Figure 4.9: Distribution of *Aspergillus* species in various markets

CHAPTER FIVE

DISCUSSION

5.1 Spice Utilization

Spices are generally used all over the world to boost the aroma and flavor of food. In this study 39 types of spices and spice blends were identified and assessed for their microbial quality. It was observed that two forms of spices were available in open air markets as natural spices and spice blends. The spice blends were mixtures of various natural spices and herbs prepared by the vendors. The natural spices comprised 54 percent of all the samples while 41 percent were spice blends and 3 percent were identified as extracts. The use of spices over the years has resulted in blending in order to develop a more flavourful composition and make them last longer as natural spices tend to become stale with time (Anderson, 2012). The availability of spices in the open air markets showed variations with paprika, Chili pepper and turmeric being the most common in all open air markets. This could be attributed to the fact that they are both locally and regionally produced and the thus the cost is relatively low (Fdii, 2013).

5.2 Microbial quality of spices

The bacterial counts in spice and spice blends were done to determine the microbial quality of these products sold in open air markets of Lusaka District. According to the guidelines set by the International Commission for Microbiology, the total bacterial count in spices must be below 10^4 cfu/g which is considered acceptable whereas the count between 10^4 cfu/g and 10^6 cfu/g is considered permissible while the bacterial count above 10^6 cfu/g is considered unacceptable (Yankey, 2014). Further no *Salmonella* is to be isolated in 25g sample (ASTA, 2017). In this study, some *Salmonella* was isolated, highlighting a serious contaminant.

The findings of this study indicated that (5.2 percent) of analysed samples were above the acceptable range for bacterial contamination whereas 2.6 percent of the samples were within acceptable standards. All in all 92.2 percent of the samples analysed were in the permissible range. The results indicate that microbial contamination in terms of quality is not constant and may be typical of a given type and blend of spice. Microbial contamination is usually affected by the

handling practices implored during harvesting, processing, transportation and storage of these products prior to use in food preparation (Bakobie *et al.*, 2017). Other sources of contamination could be environmental conditions prevailing especially in open air markets (UNIDO, 2017; Garbowska *et al.*, 2015) where personal sanitation and hygiene are not to standard.

Sarika and Shikha, (2014) suggest that inherent characteristics in some plants including natural spices may contain antimicrobial compounds such as carvacrol, camphene, piperine and thymol whose presence can exert the microflora inhibiting effect (Burt, 2004) and these may affect some spices as they may show a low Bacterial count. The absence of microbes and low microbial count of some spices could also be attributed to treatment and other processes applied during production. The presence of *E. coli* that has been identified in this study is indicative of faecal contamination. Furthermore coliform counts were also enumerated. Enterobacteriaceae counts are used more generally as an indicator of hygienic quality rather than of faecal contamination and therefore say more about general microbiological quality than possible health risks posed by the product (Adams and Moss, 1995). These findings can be attributed to various other factors such as environmental conditions, handling, and storage (Bakobie *et al.*, 2017). Some spice and spice products had relatively high microbial content with over 90 percent falling in the bacterial count range of 10^4 - 10^6 cfu/25g. Investigation results have been reported by different authors (Gabowska *et al.*, 2012; Witkowska *et al.*, 2011; Pafumi, 1986) where significant variations of microbial contamination of spices were demonstrated. Witkowska *et al.*, (2011) demonstrated high microbial contamination levels greater than 10^6 cfu/g of the spices examined that included ginger, dill and parsley while Banerjee and Sarkar (2003) recorded the total count of bacteria to be greater than 10^6 cfu/g in over 50 percent of samples. The International Commission on Microbiological Specifications for Foods (ICMSF, 1974) set up maximum limits of 10^6 , 10^4 , 10^4 and 10^3 cfu of total aerobic mesophilic bacteria, yeasts and molds, coliforms and *E. coli*, respectively, per gram spice.

Bacterial organisms identified from spice and spice blends in this study included contamination indicator microorganisms which may be significant microorganisms in causing foodborne infections. These are *Klebsiella*, *Citrobacter*, *Pseudomonas*, *Candida*, *Enterobacter*, *Streptococcus* and *Escherichia coli*. Other organisms include; *Staphylococcus*, *Bacillus*, *Salmonella* and *Listeria*. These results indicate that spices sold in open air markets of Lusaka

district contain a high load of bacteria that is capable of resulting in infections such as food poisoning, respiratory diseases and tooth cavities.

Staphylococcus species of bacteria are considered the most important causes of diseases in the world among the reported food borne diseases (Ananou *et al.*, 2007; Zhang *et al.*, 1998). It is also considered as a persistent cause of gastro enteritis worldwide especially in third world countries (Vora *et al.*, 2007). The high presence of *E. coli* and *Staphylococcus* is an indication of poor personal hygiene practices during spice preparation, handling, transportation, storage or packaging and indeed at point of sale (Kari *et al.*, 2015; Elmali and Yaman, 2005).

An observation was made during the purchase of these spices where traders were handling spices with bare hands. Further, the spice products were exposed to environmental conditions such as dust. Contaminant microorganisms could have come from food handlers as reported by Bukar *et al.*, (2009) or the utensils, air and even from the ingredients themselves as reported by Frazer and Westhoff, (2006). With such contamination spices may be cited as a source of contamination for processed products.

Salmonella spp were determined in 2.6 percent of the analysed samples. The bacterium is capable of surviving in products that have low water activities for a longer time (Zwaifei and Staphan, 2011) hence the need for modern treatment methods. *Salmonella* spp was isolated from rice spice sample collected from Tuesday Market in Chilenje sub district.

Listeria spp were also in 2.63 percent of the samples analysed. Listeriosis outbreaks are usually associated with meat and meat products. Kara, (2015) in his study of Turkish spices echoed that that contamination of spices can have an effect on the end product quality and may cause toxicity upon consumption in humans (Elmali and Yaman, 2005) as well as cause spoilage and increase the microbial load in foods to which they are added. Abou Donia, (2008) examined the spices in Egypt and established the high presence of fungi. The results for fungi detected in this study indicated three species of fungi to be involved and these were identified as *Candida*, *Aspergillus* species and *Mucor* species. The most prevalent species were *Mucor* which were found in 91 percent of the total samples whereas that of *Aspergillus* was 60 percent. As for *Mucor*, 56 percent

of all the samples analyzed indicated its presence. This finding is similar to the findings of Sumanth *et al.*, (2010). This is because spices and spice products can be exposed to fungal contamination during processing, storage, and transportation (Dimic *et al.*, 2008). The high prevalence of *Mucor* fungus is a concern. This is because these fungi are able to cause life threatening invasive fungal infections especially in immune compromised individuals. Vallambhaneni *et al.*, (2015), indicated that quantifying the public health burden is challenging especially in the absence of specific data. The presence of fungi in the spices and spice products indicate that these spices and spice products are exposed to environmental contaminants which may be of significance to public health. The mode of food preparation that involves cooking may reduce the risk of any pathogen or bacteria transmission.

As many other agricultural commodities, spices are exposed to a wide range of environmental microbial contamination during collection, processing, and in the retail markets by dust, waste water, and animal and even human excreta (De Boer *et al.*, 1985). Contaminated spices may cause a microbiological problem, depending on the end use (Van doren *et al.*, 2013). Cuisines that incorporate spices may pose a risk to public health because they are often added to foods that undergo no further processing or are eaten raw. Spices are the principal source of spore forming bacteria in large volumes of foods, such as soups, casseroles, stews and gravies produced by catering establishments; under favourable conditions, they germinate and multiply to infective and toxic levels (Pafumi, 1986).

CHAPTER SIX

CONCLUSION AND RECOMENDATIONS

6.1 Conclusion

1. Spices are widely and traditionally used in food production and consumption.
2. This study established that the microbial load of spices and spice products sold in open air markets in Lusaka district is high.
3. The spices and spice products may contain faecal contaminating indicator microorganisms as well as organisms that may be potentially pathogenic.

6.2 Recommendations

1. Locally produced spices could be produced under strict hygienic conditions as mandated by the food and drugs act, the public health act and all other applicable legislation.
2. To further apply principles such as HACCP in all processes from production leading to consumption to preserve quality.
3. Spices should be subjected to steam treatment, dry heat method or irradiation where practical in order to reduce the microbial load.
4. Ensure that sanitary facilities are adequately provided in the markets for market traders and ensure that health education is provided to all traders involved in the production in the production, handling, supply and sale of spice and spice product
5. Further, regular checks must be conducted for microbiological and contaminants surveillance and control along the supply chain beginning from the imports entry points.
6. Work is recommended in establishing the serological identification of microorganisms present in spices and their antimicrobial susceptibility.
7. Samples whose results show contamination higher than the legal thresholds will be handed to the local authority for appropriate action.

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APPENDICES

Appendix 1

Bacteria identified from the spices and spice blends collected from the sampling markets.

*Sample Name	Bacteria isolated from Sampling Point (Open Air Markets)				
	Mutendere	Soweto	Chawama	Tuesday Market	Total Number of samples Collected
1. Turmeric	<i>Salmonella</i> <i>Enterobacter</i> <i>Klebsiella</i>	<i>E. coli</i> <i>Streptococcus</i> <i>Bacillus</i>	<i>Klebsiella</i> <i>Citrobacter</i> <i>Staphylococcus aureus</i> <i>Streptococcus</i> <i>Enterobacter</i> <i>Bacillus</i>	<i>Klebsiella</i> <i>E. coli</i>	8
2. Black Pepper	<i>Staphylococcus</i> <i>Pseudomonas</i> <i>Streptococcus</i> <i>E. coli</i> <i>Enterobacter</i> <i>Klebsiella</i> <i>Bacillus</i>			<i>E. coli</i> <i>Staphylococcus</i> <i>Bacillus</i>	4
3. Paprika	<i>E. coli</i> <i>Enterobacter</i> <i>Citrobacter</i> <i>Bacillus</i> <i>Staphylococcus aureus</i> <i>Pseudomonas</i> <i>Streptococcus</i>	<i>Citrobacter</i> <i>Enterobacter</i> <i>E. coli</i>	<i>Staphylococcus aureus</i>	<i>Bacillus</i> <i>Klebsiella</i> <i>Enterobacter</i> <i>Salmonella</i>	8

4. Curry	<i>Bacillus</i> <i>Staphylococcus aureus</i> <i>Candida</i>		<i>E. coli</i> <i>Staphylococcus aureus</i> <i>Citrobacter</i> <i>Bacillus</i>		4
5. Cinnamon	<i>E. coli</i> <i>Staphylococcus aureus</i> <i>Staphylococcus</i> <i>Bacillus</i>	<i>Bacillus</i> <i>Staphylococcus aureus</i> <i>Klebsiella</i> <i>Enterobacter</i>			4
6. Chili Pepper	<i>E. coli</i> <i>Staphylococcus</i> <i>Citrobacter</i> <i>Enterobacter</i> <i>Pseudomonas</i> <i>Entermedias</i> <i>Bacillus</i>	<i>Bacillus</i> <i>E. coli</i> <i>Pseudomonas</i>	<i>Candida</i> <i>Bacillus</i> <i>Streptococcus</i> <i>Pseudomonas</i> <i>Staphylococcus</i> <i>E. coli</i>	<i>Citrobacter</i> <i>Enterobacter</i> <i>E. coli</i> <i>Staphylococcus</i>	8
7. Chicken	<i>Enterobacter</i> <i>Staphylococcus</i>		<i>Bacillus</i> <i>Klebsiella</i> <i>Staphylococcus</i>		4
8. Steak and Chops		<i>Bacillus</i> <i>Klebsiella</i> <i>Staphylococcus</i>		<i>Bacillus</i> <i>Staphylococcus</i> <i>Enterobacter</i> <i>Salmonella</i> <i>Candida</i>	4
Total samples					44

*These samples were similar spices /spice blends from different market

Appendix 2

A summary of Bacterial organisms identified from each spice and spice product type collected from single markets

SN	Sample Name	Bacteria Isolated	Total Number of samples Collected
1.	Garlic	<i>E. coli</i> <i>Bacillus</i> <i>Streptococcus</i>	2
2.	Ginger	<i>Bacillus</i> <i>Staphylococcus aureus</i> <i>Klebsiella</i> <i>Enterobacter</i>	4
3.	Ranger Biltong	<i>Candida</i> <i>E. coli</i> <i>Bacillus cereus</i> <i>Staphylococcus aureus</i>	1
4.	Corriander	<i>Streptococcus</i> <i>Staphylococcus aureus</i> <i>Pseudomonas</i> <i>E. coli</i> <i>Listeria</i>	2
5.	Fish Seasoning	<i>Citrobacter</i> <i>Enterobacter</i>	2
6.	Samoosa	<i>Klebsiella</i> <i>E. coli</i> <i>Bacillus</i>	1
7.	T.Bone	<i>Bacillus</i> <i>Klebsiella</i> <i>Staphylococcus</i>	2
8.	Shawama	<i>Mucor</i> <i>E. coli</i> <i>Staphylococcus</i> <i>Bacillus</i>	2
9.	Beef Sausage	<i>Bacillus</i> <i>E. coli</i> <i>Staphylococcus</i>	1
10	Brown Onion Soup Powder	<i>Bacillus</i> <i>Klebsiella</i> <i>Citrobacter</i> <i>Enterobacter</i>	2
11	BBQ	<i>Bacillus</i> <i>Staphylococcus aureus</i> <i>Streptococcus</i> <i>Pseudomonas</i>	1

		<i>Klebsiella</i>	
12	Chip Sprinkle	<i>Salmonella</i>	2
13	Gravy	<i>Candida</i> <i>Bacillus</i> <i>Staphylococcus</i>	2
14	Beef Stew	<i>Klebsiella</i> <i>E. coli</i> <i>Bacillus</i> <i>Streptococcus</i>	2
15	Spice For Rice	<i>Bacillus</i> <i>Enterobacter</i> <i>Staphylococcus</i> <i>Citrobacter</i>	2
16	Amchur Powder	<i>Klebsiella</i> <i>Salmonella</i> <i>Candida</i>	2
17	Sambhar Masala	<i>Candida</i>	2
18	Aromat	<i>Bacillus</i> <i>Candida</i>	1
19	White Pepper	<i>Klebsiella</i> <i>pseudomonas</i> <i>E. coli</i> <i>Bacillus cereus</i> <i>Streptococcus</i>	2
20	Paneer Tika	<i>Klebsiella</i> <i>Enterobacter</i> <i>Bacillus</i> <i>Citrobacter</i> <i>Staphylococcus aureus</i>	1
21	Kitchen King	<i>Candida</i> <i>Bacillus</i> <i>Staphylococcus aureus</i>	1
22	Biryani Masala	<i>Candida</i> <i>Bacillus</i> <i>Proteus</i> <i>Enterobacter</i>	2
23	T Bone	<i>Aspergillus</i>	2
24	Garam Masala	<i>Proteus</i> <i>Staphylococcus aureus</i> <i>Bacillus</i> <i>Enterobacter</i>	2
25	Cumin Powder	<i>Bacillus</i> <i>Candida</i>	2

26	Fenugreek Powder	<i>Candida</i>	1
27	Aarchur Masala	<i>Proteus</i> <i>Staphylococcus aureus</i> <i>Streptococcus</i> <i>Bacillus</i>	1
28	Mustard Seed Powder	<i>Candida</i> <i>Bacillus</i> <i>Streptococcus</i>	2
29	Cayenne Powder	<i>Klebsiella</i> <i>E. coli</i> <i>Bacillus cereus</i> <i>Streptococcus</i>	1
30	Mix Lemon	<i>Mucor</i> <i>Candida</i>	2
31	Clove	<i>Proteus</i> <i>Bacillus</i> <i>Candida</i>	2

Appendix 3

A summary of Fungi identified from each spice and spice blends collected from single markets

	Sample Name	Fungi isolated from Sampling Point(Open Air Markets)							
		Mutendere		Soweto		Chawama		Tuesday Market	
		<i>Mucor</i>	<i>Aspergillus</i>	<i>Mucor</i>	<i>Aspergillus</i>	<i>Mucor</i>	<i>Aspergillus</i>	<i>Mucor</i>	<i>Aspergillus</i>
1.	Turmeric	+	-	+	-	+	+	+	+
2.	Black Pepper	+	-					+	-
3.	Paprika	+	-	+	+	+	-	+	-
4.	Curry Powder	+	-			+	-		
5.	Cinnamon	+	-	+	-				
6.	Ground Chilli	+	-	+	-	+	-	+	-
7.	Chicken	-	-			+	-		
8.	Steak and Chops			+	+			+	+
9.	Garlic	-	-						
10.	Ginger	+	-	+	+				
11.	Ranger Biltong			+	-				
12.	Coriander			+	+			+	-
13.	Fish Seasoning			+	-				
14.	Curry Powder			+	+			+	+
15.	Samoosa			+	+				
16.	Shawama			+	+				
17.	Beef Sausage							+	+
18.	Brown Onion Soup Powder							+	+
19.	BBQ							+	+
20.	Chip Sprinkle			+	+				
21.	Gravy			+	+				
22.	Beef Stew							+	+

23.	Spice For Rice			+	+				
24.	Amchur Powder							+	+
25.	Sambhar Masala							+	+
26.	Aromat							+	+
27.	Black/White Pepper	+	+						
28.	Paneer Tika							+	+
29.	Kitchen King							+	+
30.	Biryani Masala							+	+
31.	T Bone			+	+				
32.	Garam Masala							+	+
33.	Cumin Powder							+	+
34.	Fenugreek Powder							+	+
35.	Aarchur Masala							+	+
36.	Mustard Seed Powder							+	+
37.	Cayenne Powder							+	+
38.	Mix Lemon							+	+
39.	Clove							+	+

