AN APPLICATION OF HAZARD ANALYSIS CRITICAL CONTROL POINTS (HACCP) IN THE PREPARATION OF TRADITIONAL OPAQUE SWEET BEVERAGE (*THOBWA*) IN BLANTYRE CITY VENDORS

MASTER OF SCIENCE IN ENVIRONMENTAL HEALTH THESIS

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UNIVERSITY OF MALAWI

THE POLYTECHNIC

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MASTER OF SCIENCE IN ENVIRONMENTAL HEALTH THESIS

By

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(BSc CD)

A Thesis Submitted to the Department of Environmental Health, Faculty of Applied Sciences, in Partial Fulfilment of the Degree of Master of Science in Environmental Health

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The Polytechnic

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DECLARATION

I, the undersigned, do hereby declare that this thesis titled '*an application of Hazard Analysis Critical Control Points (HACCP) in the preparation of traditional opaque sweet beverage (thobwa) in Blantyre city vendors*' is my own original work which has not been submitted to any other institution for the attainment of a degree. Where other people's work has been used, acknowledgements have been made.

Name : Phillimon Peter Phiri

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Date : _____

CERTIFICATE OF APPROVAL

We, the undersigned, certify that we have read and hereby recommend for acceptance by the University of Malawi a thesis titled '*An application of Hazard Analysis Critical Control Points (HACCP) in the preparation of traditional opaque sweet beverage (thobwa) in Blantyre city vendors.*

Main Supervisor : _____

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Date : _____

Dean- Postgraduate : _____

Signature : _____

Date : _____

Head of Department : _____

Signature : _____

Date : _____

DEDICATION

I dedicate this thesis to my late father, who couldn't wait for the success of his children before answering God's call, my mother Nellie, wife Linly and children; Chisomo, Chiyanjano and Chiyamiko.

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First, I would like to thank God almighty for giving me the strength and courage plus wisdom to complete this work.

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ABSTRACT

While the selling of a traditional opaque sweet beverage locally known as *"thobwa"*, is on the rise in all the cities of Malawi, diarrhoeal diseases are also on the rise. This is more common among low-income earners. The rising cases of diarrhoea might be due to several factors including food handling and preparation. *thobwa*, especially the locally prepared is one of the high-risk drinks due to its preparation stages, and the purpose of this study was to assess the levels of contamination in this locally prepared *thobwa* and how it can be minimised by following the Hazard analysis critical control point (HACCP).

This study employed a descriptive cross-sectional method involving both qualitative and quantitative data. The methods involved the use of structured questionnaires to collect data. The questionnaire was administered to a population of 30 who were doing *thobwa* selling business around Makata Industrial Area. Observation checklist and laboratory investigations were also used to a randomly selected 14 respondents from the 30, to capture the stages in the *thobwa* preparation, during the collection of laboratory samples. These 14 were followed up in all the stages of *thobwa* preparation, including taking samples at every stage of preparation. These samples were *thobwa* and the water used in *thobwa* preparation, hand swabs of food handlers plus swabs from bottles used in packaging the *thobwa*. Furthermore, additional notes were being taken during observation in the process of preparing the *thobwa*, including behaviours which might affect the quality of the *thobwa*, either negatively or positively. In addition to the samples, following the HACCP principles, the study identified critical points where *thobwa* gets contaminated in the process of preparation, so that control measures are put in place to control or minimise the contamination levels.

Of the 37 samples of *thobwa* processed within 48 hours, 56.7% (n=21) had *E. coli* and 67% (n=25) had *Salmonella* while 75% (n=28) had *Staphylococcus* growth. In terms of levels of contamination, *Staphylococcus* was more present in most samples collected. Furthermore, out of the 23 samples of water analysed, 69% (n=16) were having positive coliforms while 30% (n=7) were having positive *E. coli*. The study further revealed that *thobwa* contamination increases at each stage of preparation for both *E. coli*, *Salmonella* and *Staphylococcus,* with positive correlation which was not statistically significant. On the HACCP, it was established that four stages constitute the critical points which are cooking, cooling, bottling, and handling in general apart from the water used at both household and selling point level. This contributes to the increased level of contamination to *thobwa*. On sanitation part, waste disposal is an issue as 66.7% of the respondents dump their wastes in the open pit while only 13.3% are putting their wastes in plastic containers and bins.

In general, the study has revealed the presence of contamination in the *thobwa* which is bought from venders, and this contamination is caused by the handling process during preparation at both household and market level. Furthermore, the study has also established the behaviour and hygiene practises done by the food handlers in the home, especially during the preparation of the *thobwa*. Furthermore, the study has also established the increased levels of contamination of the *thobwa* as the stages in preparation are advancing. Following the HACCP principles, it has also been revealed in the study that there are specific areas and specific points when contamination can take place during the preparation of *thobwa*.

ABBREVIATIONS AND ACRONYMS

CCP	Critical Control Point
CI	Confidence Interval
COM	College of Medicine
CV	Curriculum vitae
EH	Environmental Health
FAO	Food and Agriculture Organisation.
HACCP	Hazard Analysis Critical Control Point
HOD	Head of Department
MBS	Malawi Bureau of Standards
MDHS	Malawi Demographic Health Survey
MICS	Multiple Indicator Cluster Survey
MOH	Ministry of Health
NHSRC	National Health Sciences Research Committee
NSO	National Statistical Office
ODK	Open Database Kit
PI	Principal Investigator
SABS	South African Bureau of Standards
SHARE	Sanitation and Hygiene Applied Research for Equity
SPSS	Statistical Package for Social Sciences
UNICEF	United Nation Children Emergency Fund
WHO	World Health Organisation
WASHTED	Water Sanitation Health and Appropriate Technology Development

CHAPTER ONE

INTRODUCTION AND BACKGROUND

1.1 Introduction

In 2017, the World Health Organization (WHO) estimated that 1.7 billion children under the age of five suffer from diarrhea each year and in terms of mortality, diarrhea was the second leading cause of death among these children. In 2016, it was the eighth cause of death among people of all ages (WHO, 2017). The disease is caused by bacteria, viruses and parasites and is transmitted through drinking contaminated water, eating contaminated food or from person to person because of poor hygiene. Africa was estimated to have the highest burden of diarrhoeal diseases at 687 disability adjusted life years per 100,000 population of all ages five (Black et al., 2010). In Malawi, 22% of the children under the age of five years were reported to have diarrhea two weeks preceding the national survey in 2015 (National Statistical Office, 2015).

The relationship between diarrhoea prevalence and food hygiene has been widely investigated especially in children under the age of five, however, limited evidence is available on the impact in people of ages above five years despite them also being affected (Kirk et al., 2012). Poor food hygiene is one of the contributors to diarrhoea prevalence. In this study, we focused on the traditional opaque sweet beverage "*thobwa"*, a ready to drink food that is sold in local markets in Malawi. This is also known as sweet beer in other countries, and locally, it is made from flour made from either of the following: germinated maize or germinated millet flour and or potato flour. Both the maize and millet flour must be well prepared. During germination, a fermentation of some kind takes place, allowing other fungus to get access to either the maize or millet flour. The maize must be pounded from the mill and the millet is crushed using the mortar and pestle or maize mill for them to be in flour form. Naturally, the germination process of the maize or millet seed helps in the production of natural live microorganisms or their metabolites which have several health benefits, including the inhibition of foodborne causing bacteria (Minamor et al., 2017). The preparation is a process which involves boiling of water, mixing the water with flour, adding millet to the mixture then cooling and then packaging the mixture. This handling process has the greatest potential of cross contamination of other foodborne microbes like *E. coli*, *Salmonella* and *Staphylococcus*. Currently, the sale of *thobwa* in informal and formal markets in Malawi and Sub-Saharan Africa is not uncommon. The drink is affordable making it the most common drink taken during lunch and given to children in homes. It has been noted that local food selling including *thobwa* selling is always high among

the low-income earners, yet incidences of diarrhoea and cholera are major public health challenges facing Malawi and other countries in the region, affecting the same category of low-income earners National Statistical Office (NSO,2017). Literature has shown that there are a lot of varying processes that *thobwa* undergoes before it reaches the end user, while also using utensils whose hygienic standards are highly questionable, making it a potential source of contamination for cholera and other diarrhoeal related diseases (Kitabatake et al., 2003). *Thobwa* has been produced and consumed in cities for years without following food safety principles. It was therefore important to assess the contamination levels and identify the critical control points for the prevention of contamination using the Hazard Analysis Critical Control Point (HACCP).

HACCP has been used to reduce food contamination especially weaning foods because of the high diarrhea burden among children less than 2 years of age (Islam et al., 2013). HACCP is a system that enables the production of safe food through the analysis of production processes, identification of all hazards that are likely to occur during food preparation, the identification of critical points in the food preparation process, the establishment of critical limits for control at those points, the verification of these prescribed steps, and the methods by which the one preparing the food and regulatory authorities the processes (Hulebak & Schlosser, 2002). The Hazard Analysis Critical Control Point (HACCP) manual was written to provide a "roadmap" for evaluating retail and food service establishments based on the application of HACCP principles. These are preventive approaches implemented by any industry in controlling food safety hazards. Using these principles during inspections will always help to assist in evaluating the effectiveness of food safety management systems implemented by industry or the local markets (Center for Food Safety and Applied Nutrition, 2006). Improving food hygiene is important in achieving good health through diarrhoeal reduction and hence assisting in achieving the Sustainable Development Goals (SDGs) (Morse et al., 2018).

1.2 Statement of the problem

It has been noted that local food selling including *thobwa* is always high among the low-income earners, yet incidences of diarrhoea and cholera are also on the rise (Department of Epidemiology, 2018) Recently, in the year 2017/18 rainy season, the country had experienced a rise in the number of cholera cases and the figures were rising at an alarming rate. A good example can be noted in the month of February. On 12th February 2018, the country recorded a total of 459 cases and on the 13th February, the figures rose to 469. On the 14thFebruary, the recorded cumulative cases were 478. If the trend is to go by, there are almost 10 new cases of

cholera registered daily in Malawian health facilities. These cumulative cases were reported in the 13 affected districts including Lilongwe and Karonga, with 7 deaths (Epidemiology Unit, 2018). This situation is worrisome especially considering that the cases are more in towns where informal food restaurants and other open markets could be highly patronised. *Thobwa* has been produced and consumed in towns and cities for years without following food safety principles. It was therefore important to assess the contamination levels of *thobwa* as is similar to other openly sold foods and drinks, where several factors may make it prone to microbial contamination. Potential microbes that can contaminate *thobwa* may include bacteria such as *E. coli*, *Salmonella species*, *Shigella species* and *Staphylococcus aureus* and identify the critical control points for the prevention of contamination using the Hazard Analysis Critical Control Point (HACCP), as it could be one of the contributing factors to the spread of foodborne diseases including cholera. The mentioned pathogens are of great importance in public health as they are mainly harboured by man in either nose or hands, hence increased potential of cross contamination by food handlers, and may easily be transmitted through local drinks, uncooked or partially cooked food and they are not supposed to be found in high quantities for some of them while others should be at zero quantity in any food substance before consumption (Umaru et al., 2014).

1.3 Significance of the study

The study helped to identify other sources of contamination for locally prepared drinks, which have been ignored for so many years and by identifying the sources, the study will also provide guidance and direction to policy makers and the public in the prevention of the foodborne diseases like cholera and diarrhoea. Apart from that, the study will also provide a better platform to decision makers in sensitising the local community especially the food vendors in the safer way of food handling and preparation with focus on *thobwa* which turns to be the highly consumed by low-income earners working in the industrial areas within the cities.

1.4 Main study objectives

- To apply HACCP in determining the points of contamination, behaviour of food handlers and levels of contamination for *thobwa.*

1.4.1 Specific objectives

1. Access the practices of food handlers during preparation, cooling, storage, transportation and selling or serving of the *thobwa*
2. Establish levels of contamination in the locally brewed *thobwa*.

3. Identify the points of contamination for the *thobwa*
4. Develop a HACCP system to reduce contamination in the *thobwa* preparation stages

1.5 Research question

1. How does HACCP help to eliminate *thobwa* contamination during preparation?
2. What is the level of contamination in the *thobwa* as it reaches the consumer at the market?

1.6 Conceptual framework

This study is trying to establish the application of HACCP in controlling the contamination levels in the preparation process of *thobwa.* The HACCP system as indicated in the Essential guide, is one of the main strategies used in the food service industry be it local or something more complex. It is a systematic set of activities used to control food production to ensure food safety and prevent changes in foodstuffs. The system is based upon the use of control practices in given production steps where there is a greater probability of occurrence of health hazards. The prerequisite programs for HACCP implementation in food industries involve several aspects of the food industry, such as physical structure and maintenance, water supply, personal hygiene, pest control, sanitization techniques and equipment used in the production process among others (Safe Food Alliance, 2019).

The proper identification of CCPs is a key issue in HACCP, because the major efforts in process control will be directed towards these steps. *Thobwa* production process is subject to a diversity of pollutants because of its handling process, and it is necessary to control the extent to which this occurs. For the practical application of the HACCP concept according to Codex Alimentarius, 7 rules must be followed which are laid down in main principles and constitute the basis for the establishment of a HACCP plan. These principles include performing a hazard analysis, determining the Critical Control Points (CCPs), establishing critical limit(s), establishing CCP monitoring system, establishing corrective action to be taken if monitoring indicates that a specific CCP is no longer under control, establishing procedures of verification to confirm a working system of HACCP and introducing a documentation system, taking into account all processes and records in accordance with the principles and their application. In the event of *thobwa,* the practises of food handlers are to be identified as they have a potential of either controlling contamination or predisposing more contamination. Then having identified

their practises, levels of contamination for *thobwa* are to be established through laboratory tests, then points of contamination are to also to be identified, which will in the end assist in developing a system for HACCP, to reduce contamination in the *thobwa* preparation stages. The critical limit is usually a measure such as time, temperature, water activity (Aw), pH, weight, or some other measure that is based on scientific literature and/or regulatory standards. The HACCP team will describe monitoring procedures for the measurement of the critical limit at each critical control point. In this study, the time taken to boil the *thobwa*, the temperature ranges and the time it takes to cool off the *thobwa* will help in measuring and identifying the critical limits so that control measures are put in place in the preparation process of the *thobwa*.

The findings of this study will further add academic and professional knowledge on *thobwa* preparation to the already existing literature since there is a gap in literature regarding the preparation of the *thobwa.* In Malawi, it is also expected that the findings of the study shall generate interest for future researchers in this field to undertake further research on the contamination levels in *thobwa* preparation.

Further findings from the study will help to improve health through better preparation of food, thereby reducing the foodborne diseases, contributing towards the achievement of SGD goal number three of good health and wellbeing, especially reducing mortality.

CHAPTER TWO

LITERATURE REVIEW

2.1. Introduction

The review of literature tries to explore the process of *thobwa* preparation, with the aim of establishing points of contamination before it reaches the market for selling and or while it is being sold to the customers.

2.2. Food contamination during preparation

One of the basic requirements in a human being is food. This food might be bought or prepared right at home. The food people eat, place them at a risk of contracting infections by food-borne pathogens. Other studies have shown that many outbreaks originate from home let alone the food which people eat. Of the many food items consumed in Malawi, nsima is on the list and it requires several ingredients for it to be prepared. According to Taulo et al., (2008) in an article titled 'Microbiological hazard identification and exposure assessment of food prepared and served in rural households of Lungwena', it is a common thing that most of the local masses are able to store their left-over food for consumption at a later time. Meaning they also consume cold food left for many hours. Furthermore, the preparation itself of such food also pose a threat in the consumption, as contamination level is also on rise during the preparation stages of the food (Taulo et al., 2008). In general, the study found maize flour to be the most contaminated food category. Thus, the flour which is used to prepare the commonly known nsima and other food items including *thobwa,* which this study was interested about. In another study conducted in Zimbabwe, Sanza which can locally be equal to nsima, was also reported to have some contamination among other foods (Taulo et al., 2008). This can as well be attributed to high levels of contamination of the flour itself, as identified in other studies. On a different note, issues of contamination are always complex in nature as most levels may require massive proof through laboratory investigations. Since time in memorial, the control and reduction of foodborne disease has not been a development priority, despite its integral role in the health and well-being of the world's population (Morse et al., 2018). There are huge levels of contamination in the food which is eaten and at times bought from the market, only that they go unnoticed just because their levels of contamination might be low to cause sickness to humans or that the human immunity is strong enough to naturally fight the contamination in the body. The same can be applicable to *thobwa,* as the preparation process itself cannot isolate it

from having huge levels of contamination. This is also in line with another study conducted by (Taulo et al., 2008), which found that most contamination levels take place by the food handlers during the preparation stage of the food (Taulo et al., 2008). For instance, if someone is peeling some vegetables, there might be transfers of pathogens from the hands to the vegetables, yet the pathogens might not cause harm just because the vegetables are cooked and in the process the pathogens might have died or maybe because the pathogens might not be in a number which might cause harm to humans. Even though food is cooked (boiled) at a temperature high enough to inactivate pathogens, post-contamination and cross-contamination that is being promoted by unhygienic food handling, and poor storage practices cause this "safely prepared food" to be unsafe, and handwashing plus food storage utensils also plays a vital role as far as food safety and hygiene are concerned.

2.3. Hand washing in relation to food contamination

Hand washing also known as hand hygiene according to World Health Organisation (WHO, 2009) has been defined as an act of cleaning hands for the purpose of removing soil, dirt, and microorganisms or other unwanted substances. This act must be done thoroughly with soap and water for a period of not less than two minutes (WHO, 2009). According to the humanitarian response of 2018, the sphere standards have also highlighted handwashing as one of the important elements in following proper hygienic principles. Sphere has further highlighted the importance of hand washing after defecation and before eating and preparing food, to prevent the spread of disease. Food handlers should have the means to wash their hands after defecation with soap or an alternative (such as ash) and should be encouraged to do so before they start their food preparation. There should always be a constant source of water near the toilet for this purpose (Sphere Association, 2018). There are no alternative means to handwashing as far as prevention of communicable diseases and hygiene related diseases are concerned and the situation is not in any way isolating food preparation including the preparation of *thobwa*.

Germs can be spread in many ways, including touching items with dirty hands, changing dirty diapers, through contaminated water and food, through droplets in the air released during a cough or sneeze, on contaminated surfaces, through contact with a sick person's body fluids. When kids and or adults encounter germs, they can become infected just by touching their eyes, nose, or mouth. And once they are infected, it is usually just a matter of time before the whole family comes down with the same illness. Some people are disease carriers, who may not fall sick but can easily transfer contamination from one person to another. Good hand washing is

the first line of defence against the spread of many illnesses including the common diarrhoea. The procedure of hand washing is simply in the following steps:

- Dipping of the hands in warm water.
- Make sure the water is not too hot for little hands.
- Use soap and lather up for about 20 seconds (antibacterial soap isn't necessary as any soap would serve the purpose).
- Making sure to clean in between the fingers and under the nails where germs like to hang out and don't forget the wrists.
- Finally, rinse and dry well.

The regular hand washing has to be made a rule in the family to stop the spread of germs and it has to be done, especially before eating and cooking, after using the bathroom, after cleaning around the house, after touching animals, including family pets, before and after visiting or taking care of any sick friends or relatives, after blowing one's nose, coughing, or sneezing and also after being outside (playing, gardening, walking with a dog, etc. (WHO, 2009). Furthermore, (Martins, 2006) investigated how frequent the food handlers can wash their hands including the utensils which are used in the food selling locations. The findings still lead to the usual challenges in the food handling process. The open food selling points in Malawi are not comparable to those in other countries like Zimbabwe and South Africa, as according to the study, most open food handlers in South Africa have been trained and are at an advanced stage on food hygiene including handwashing (Martins, 2006). In Malawi, food handlers of *thobwa*, as with many other meal or food item which are seen being sold in open markets, gates of industrial companies, where a lot of low-income workers can patronise for their daily meals, still have a compromise in standards of food safety and hygiene. What is not clear with *thobwa* as compared to other food items, is the level of hygiene which is put in place during the preparation, packaging, storing and transportation of the commonly patronised drink.

2.4. Preparation of *thobwa*

When it comes to preparation of *thobwa*, no literature has clearly been documented on how the preparation is done, even though there is a rich history of it being among the highly patronised food and drink on the local scene, as documented by (Morse et al., 2018). Further to *thobwa* specific preparation, first, there is need for organising requirements. Some of the important materials during preparation include but not limited to maize flour, millet flour, water, and buckets for boiling water. Both the maize and millet flour must be prepared. Maize pounded from the mill while the millet is first allowed to germinate before being crushed, to turn into

flour form. The preparation is a process which involves boiling of water, mixing the water with flour, to make a thick porridge. When the porridge is left to cool, before it is completely cooled, Millet flour is added to the porridge to turn it into the *thobwa*. When completely cool, packaging follows.

According to Staff Writer (2012) it all starts with boiling water then mix with maize flour until it becomes thick like porridge. After the porridge boils enough for almost twenty minutes, the fire is put off, allowing the porridge to cool off for almost 30 minutes. When the porridge begins to cool off, millet flour, made from the germinated millet seed, which has been pounded in a mortar or maize mill, is added to the cooling porridge before it completely cools off. The millet is slowly added to the porridge while stirring with a wooden spoon to allow the concentration to be fully mixed up. After the mixing is done, the mixture is transferred in another pot and let it stand for a whole day. Normally this must take the whole day or night as it allows fermentation to take place as the millet flour added was made from germinated millet seed. On the next day, depending on locations or cultural background, the *thobwa* must be re-boiled for five minutes and let it cool before sugar is added to it and then stir until it is completely dissolves, then served or packed in bottles (Staff Writer, 2012)

Due to absence of enough literature on the local scene, it might not be clear on specific stages of contamination to the *thobwa* but the cooling process might be one of them, apart from the equipment used in the storing the *thobwa,* and the utensils used in mixing the millet flour, sugar and other ingredients. Simply based on the study on food handling and contamination done is South Africa, there is evidence enough that contamination can take place at any level of food preparation and handling (Martins, 2006). In Turkey, based on the study also conducted, it revealed that food handling leaves a lot to be desired as far as handling and contamination are concerned, as the study directly revealed the relationship of food preparation in kitchen and those families without a kitchen, versus the food levels of contamination (Takanashi et al., 2009). On the local level, no studies have been conducted as regards to the same.

2.5. Cooking

The cooking process of *thobwa* begin with maize flour porridge preparation which must be left boiling for nearly an hour or two depending on level of energy (fire). After that process of porridge making, while still hot but off fire, the maize flour porridge is mixed with germinated millet flour which is poured into the porridge, mixed slowly while stirring for complete dissolving, to allow concentration and mixing of the two. While mixing, the thick porridge turns slowly into slight watery but still think enough and then it is left open for cooling. The entire

process above must be done either in the same pail which was put on the fire or a bigger basin (bucket) which could allow free mixing of the flours while off fire. In the process of cooking and flour mixing, some contamination may take place as the flour is at times mixed with hands which are not properly washed or at times the flour itself might be contaminated without the owner actually being aware of the levels of contamination, as it happened in another scenario in Thyolo (Kamwendo, 2017). Sometimes even the use of the scooping spoon may also compromise hygiene standards as the scooping spoon may have also been contaminated due to storage and cleaning mechanisms.

2.6. Storage

The storage of *thobwa* is coming in from the stage of sugar mixing, which is done either in the open bucket or pail as it is left open to cool, for a period of not less than five hours which allows cooling to take place also. Mostly, the cooling is done overnight. To this effect, some creatures like lizards, rats, cockroaches and even household pets like cats may gain access to the open bucket, which is storing the *thobwa,* making it contaminated. In this case, storage is specifically looking at the buckets which keep the *thobwa*, handling of utensils and all the related raw materials used in the process. There are also some bottles and cups, which are used during the selling of the *thobwa* to the end users. For those using bottles, they are either used for bottling and returned to the seller while at times the bottles might be taken away by the buyer as it looks to be a convenient package for a drink while somebody is moving or doing some pieces of work. The care of these cups and bottles used, can also be another source of food contamination and pause a great risk to the end users. Research has also not been done enough to appreciate the contamination levels at this stage.

2.7. Bottling

The bottling process is another area requiring special explanation as regards to *thobwa* issue. Even though literature is also silent in *thobwa* bottling, local village experience which is not documented has provided direction in the bottling process. To begin with, the bottles used in the packaging of *thobwa* are sourced differently. Commonly, they are already used bottles which were either meant for water or drink and have been thrown away by the end user of the drink or water. Children are the most common source of these bottles as they collect them in abundant and sell at a small reasonable price to those who recycle them for *thobwa* selling. Some of these bottles are sourced from friends who collect them from workshops where bottled water was being served to chill the participants while the meeting or training is in progress. Once these bottles are collected, they are washed with water in a bucket, mostly together with

cups used for distribution of *thobwa* to the customers during a normal business environment. The condition of the water used for cleaning the bottles, varies from one household to another, but mostly, this water is just collected from the water source, without any treatment to it and turns to handle a lot of bottles before replaced with clean water. Once they are cleaned, they are ready to be filled with *thobwa* and maybe stored at a cool place including the fridges. When bottles and cups have been used, they are thrown into a bucket of water where they are simply rinsed and make them ready for the next customer. This process is bigger risk as there is sugar added to the *thobwa*, which can be a very good environment for the growth of microorganisms.

2.8. Hazard Analysis Critical Control Point (HACCP)

The Hazard Analysis Critical Control Point (HACCP) manual was written to provide a "roadmap" for evaluating retail and food service establishments based on the application of its principles. HACCP is a short form for hazard analysis critical control point. This is defined as an internationally recognised management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement, and handling, to manufacturing, distribution and consumption of the finished product (Safe Food Alliance, 2019). These are preventive approach implemented by any industry in controlling food safety hazards. Using these principles during inspections will always help to assist in evaluating the effectiveness of food safety management systems implemented by industry or the local markets (Centre for Food Safety and Applied Nutrition, 2006). Evidence has shown that by simply following the HACCP principles, there is a great chance of reducing contamination in the food industry. For example, **i**n the scenario of South Africa, HACCP principles are implemented by the South African Bureau of standards (SABS) and this **is** one of the major reasons as to why contamination of meat and maize flour was found to be very low and at times almost insignificant due to the HACCP principles which the food handlers are able to follow, and thus according to a study done by (Martins, 2006), in a paper titled ‘Improving street food vending in South Africa: Achievements and lessons learned’ prepared by the International Union of Microbiological Societies presented at the FAO/WHO Regional Conference on Food Safety for Africa in Harare, Zimbabwe, in 2005. This paper concluded that the results of a study conducted on street food vending in Bloemfontein found that, overall, the microbiological quality of foods from which samples were taken was within acceptable safety limits. This was all reached because of the reason that the venders can follow proper hygienic practices in their food handling and preparation. It was also undeniable fact that street food vendors according to the said study, depends on vending for their livelihood and that their customers appreciate their trade. According to HACCP, there are five factors

which contribute directly to food safety concerns within retail and food service establishments and are collectively termed by the Food and Drug Agency as “foodborne illness risk factors.” These risk factors include Food from Unsafe Sources, inadequate Cooking, improper holding temperatures, contaminated equipment, and poor personal hygiene (Centre for Food Safety and Applied Nutrition, 2006). Most open food outlets have a biggest problem on contaminated equipment and poor personal hygiene as being the highest risk factors but following these factors and addressing each one as important is of utmost importance in the way to reduce contamination of food in open markets.

CHAPTER THREE

RESEARCH METHODS

3.1. Research Design

This study employed a descriptive cross-sectional design which involved collecting, analysing, and integrating both qualitative and quantitative data. The quantitative (e.g. experiments, surveys) methodology involved the use of structured questionnaire to collect demographic data of the respondents using close ended and a few open ended questions (Skills You Need, 2021). The questionnaire was administered to the 30 respondents, which was the total population of *thobwa* sellers around Makata industrial area, with help of one research assistant. Observation checklist was administered to 14 randomly selected households, from the population of 30, at both selling point level and at household level during *thobwa* preparation and collection of samples for laboratory investigations. To begin with, the research team, walked around the industrial area, to populate all *thobwa* sellers around and identified 32 sellers. Looking at the total population, sampling was not done but rather to take the entire population. During administration of the questionnaire, two respondents could not be traced, hence the remaining of 30 as total respondents to the study. The design is presented in *Figure 1* below.

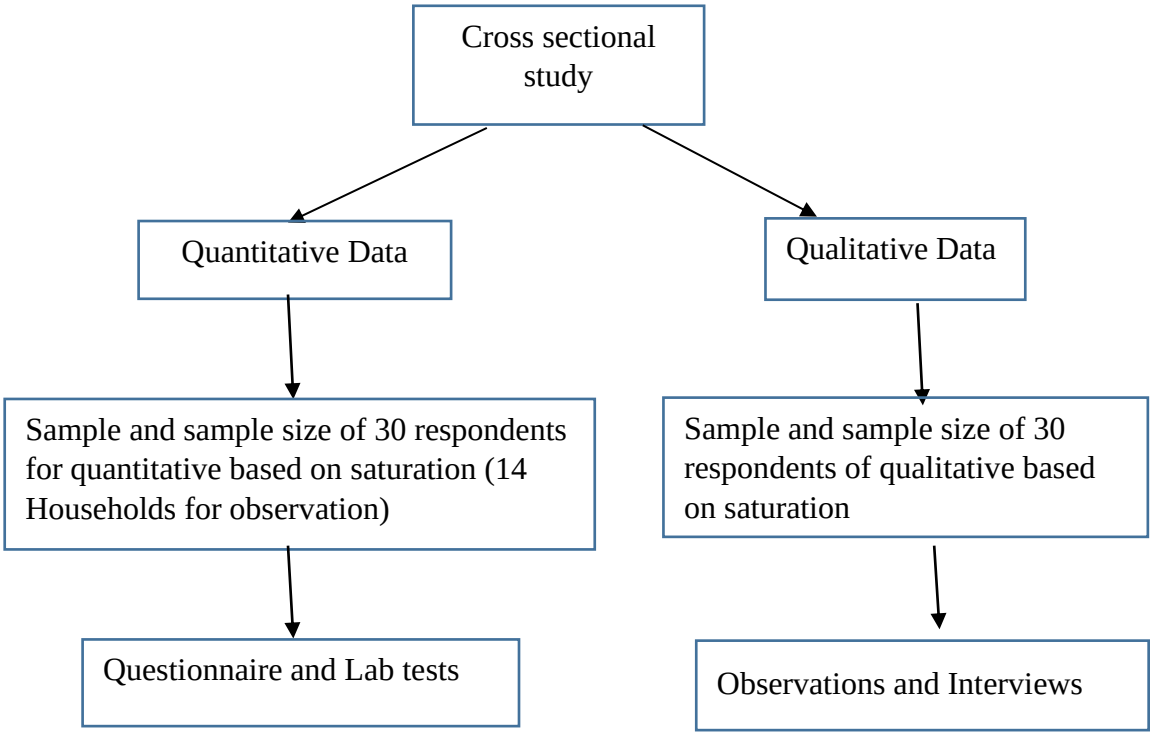

Figure 1: Study design flow diagram

3.2. Study population

The study was conducted in Blantyre city, targeting 30 food venders who were found selling *thobwa* around Makata industrial area within Ndirande township. These were the entire population of *thobwa* sellers conducting their business around Makata industrial area.

3.3. Sample size

This study employed 30 respondents who were patronising the industrial area, to serve the industrial labours with *thobwa* business. The area of Makata industry was chosen considering that it is an industrial site, which meant an increased level of casual labourers, who are potential customers for *thobwa.* and it could be easy to identify and follow up with them.

For the quantitative part of the study, 14 respondents were selected randomly from the- already identified 30 *thobwa* sellers, to partake the observation in the preparation of the *thobwa*, including collection of samples for further laboratory investigations to establish the levels of contamination in the *thobwa* that is sold to industrial workers around Makata industrial area. The follow up to these 14 included visiting in their homes during evening, around 6pm to 8pm, to appreciate the preparation stages of the *thobwa* and collection of samples in the preparation stages. Then the same households visited during the morning to take another sample, followed by another sample from the same household but collected at market point where selling was taking place.

3.4. Sampling technique

The most conveniently available *thobwa* sellers around Makata industrial area were used for the study, to be respondents for the questionnaire and a computer-generated random selection was used from the conveniently identified 30, to come up with 14 respondents, who were visited at their household for observation during the *thobwa* preparation at household level, where samples were also collected. This area was chosen as it had the highest number of *thobwa* sellers, who reside in Ndirande residential area. Furthermore, Makata being an industrial area, has the highest number of casual labours (low-income earners) who mostly eat in informal restaurants, where *thobwa* sellers are also found.

A total of 181 different samples for laboratory investigations were collected in different points from the same *thobwa* producers and sellers, to establish sources of contamination. In this case, at each identified point, three different samples were collected for each test. For *thobwa*, the samples were collected at different times, from the same source as cooking is mostly done in the evening. That is the time, when the first samples were being collected and then in the

morning of the following day, another sample was being collected as the *thobwa* had cooled off, while the last sample was being collected at the selling point, from the same producer, already packaged in the bottle. These samples were being collected for testing of both *E. coli*, *Staphylococcus* and *Salmonella*. In addition to the *thobwa* samples, hand swabs from the food handlers were also collected plus swabs from bottles which are used to package the *thobwa*. The bottle swabs were collected twice. First from the clean bottle at household level before packaging was done, then another swab at selling point level, after the bottle has been used.

3.5. Data and sample collection

Data was collected using an electronic tool called (Open Data Kit) ODK, a programming tool as on Appendix 1 were the questionnaire, administered electronically using *ODK* and an observation guide as appended on Appendix 2. The observation guide helped in addressing some specific areas which could not be addressed by the main respondent questionnaire. An interview guide also formed part of the data collection tools. For quantitative, in addition to the questionnaire, there were also some samples which were collected for laboratory analysis by the researcher, with support from the laboratory team of college of medicine and polytechnic. The question guide included both close and open-ended questions (semi structured and structured questions). It was formulated in English but was translated in Chichewa for easy communication with the participants. The interview method was chosen because it had a distinct advantage in that it enabled the researcher to establish a rapport with participants and gain their cooperation which later enabled collection of rich information during the study.

On the part of samples, they were collected in the identified 3 main stages of *thobwa* preparation, where contamination could potentially take place. These were identified in the preparation of the *thobwa* and these include the time when the *thobwa* was cooked for the last time, when the *thobwa* is left to cool before put in bottles and when the *thobwa* is being sold in bottles at the market. The collection process involved visiting the household during the evening, as that is the time when *thobwa* is prepared at home, and then another sample from the same source was collected in the morning of the following day, before *thobwa* is packaged in bottles, then another sample from the same source was taken late in the afternoon, around lunch hour or thereafter, and that *thobwa* was already packaged in the bottle, being sold to potential customers. Furthermore, water samples were also collected with empty bottles which were used for packing the *thobwa* for consumer convenient at market level. These water samples were collected from the water which is used for both drinking at household level plus cleaning of utensils. Apart from that, hand swabs were also collected from the *thobwa* producers/sellers. These swabs were taken during the time when *thobwa* preparation was at the stage of mixing

with millet flour, as this is the stage when *thobwa* has been cooked last and once the hands are contaminated, it was easy for transferring contamination to the *thobwa*.

3.6. Sample analysis

Laboratory analysis was done on all the collected samples, to establish the following.

- *E. coli* contamination in *thobwa* and surfaces of bottles and hands at packaging stages, to establish the role of food handlers in *thobwa* contamination and the role of recycled bottles used for packaging the *thobwa*, in spreading contamination.
- *Salmonella* contamination in *thobwa* at different stages of preparation and surfaces of recycled bottles and hands of food handlers in the preparation and packaging of the *thobwa*.
- *Staphylococcal* contamination in *thobwa* at different stages of preparation, water used in cooking the *thobwa* and cleaning the bottles and cups, and the surfaces of bottles used in packaging, plus the hands of food handlers during cooking and packaging of the *thobwa*.

Both samples collected, were incubated for 24 to 48 hours, in the laboratory for growth of *E. coli*, *Salmonella* and *Staphylococcus.*

The process of analysis of samples in the lab was done according to existing standard protocols. "Most Probable Number and Colilert test" were done for water samples (*E. coli* and total coliforms) while *thobwa* samples used Petrifilm count plates for *E. coli*, *Staphylococcus* and *Salmonella* as already present in Malawian laboratories (IDEXX Laboratories, 2018). The protocols are also annexed in Appendix 7.

3.7. Data analysis

Data from ODK were converted into SPSS for Windows v20.0 for cleaning using a utility called Stat transfer. Data labels and value labels were attached to the variables and data values to guide data analysis. During data cleaning, sorting was done to identify and edit or remove out of range entries, to identify and edit or remove duplicate entries, to identify and insert skipped entry frequencies and to identify and edit or remove invalid and inconsistent entries. After data management in SPSS, the output was further formatted in Microsoft Excel 2016 where descriptive statistics in the form of tables, graphs and charts were used to analyse the data. Cross tabulation was conducted in SPSS to establish relationships of contamination of *thobwa* against some practises in the preparation stages, thereby identifying the critical control points in the preparation stages of *thobwa*.

3.8. Ethical approval

For both quantitative and qualitative, ethical considerations were put in place, as the respondents of the study were not asked their names and their identification details were also not documented in any way or published at any site. On the part of the samples collected for laboratory analysis, the samples were given numbers to represent sites of collection rather than identifying them with the name of the source as it could have jeopardised the ethical considerations of making the results anonymous and not directly connected to the respondents. In general, ethical issues relating to the research participants like their consent, sensitive information and anything that could bring harm to participants were all taken into consideration as per participant information sheet and consent form which are appendix 4 and 5 respectively. On the part of the study team, ethical issues were considered like not recording their personal details for identification but rather use of identification codes when collecting data and samples, use of appropriate research methodology and correct reporting. Clearance to carry out research on human subjects was also obtained from the National health sciences research and ethical committee (NHSRC) under ministry of health, as part of ethical consideration. Protocol #18/04/2018 was referred and an approval # 2018 was provided for the study to be conducted. *Appendix 6* is attached, titled *letter of approval for ethical clearance of study on human subjects with the title "an assessment on the levels of contamination of thobwa and how it can be minimised using the HACCP system in Blantyre city vendors"*.

CHAPTER FOUR

STUDY RESULTS

4.1. Demographic characteristics of the respondents

A total of 30 respondents were recruited into the study, with 43.3% (n=14) of those respondents observed further for collection of samples. These samples included *thobwa* and other materials used in the preparation and processing of the same, before reaching the consumer to establish levels of contamination in the *thobwa* which is sold in bottles in the industrial area. Out of the total respondents, most of them 83.3% (n=24) were females. Of those women who responded to the questionnaire, it was further noted that the majority did not go beyond primary school as only 33.3% (n=12) of the respondents reached secondary level of education. Furthermore, 13.3% (n=5) never went to school. Additionally, 36.7% (n=13) of the *thobwa* sellers were heads of their households and over 80% (n=28) of them rely on *thobwa* selling as the only business to sustain their living while 26.7% were either employed male or female to sell the *thobwa*, identified as other male or female in the Figure 2 below.

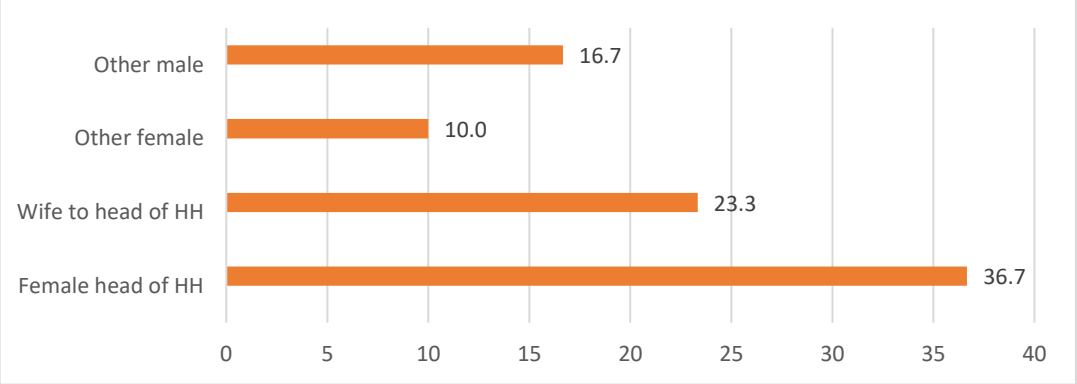

Figure 2: Respondents Demographic characteristics in percentage.

4.2. *Thobwa* preparation

It was noted that *thobwa* preparation stages don't differ from one household to another as the process begins with prior processing of the maize flour and germinated millet flour then the routine preparation of porridge. When the porridge is ready (when it turns thick and brownish in colour), it is removed from the fire, let it cool for some minutes, mix with millet flour while it is being cooled and let it cool completely. When cooling is complete, it is when there is a slight difference in *thobwa* producers as some could re-boil for the second time after millet flour is added while others do not re-boil the *thobwa*, as summarised in the Table 1 below.

Table 1: Stages of thobwa preparation

Process steps in preparation of the *thobwa*	**percentage**
Method 1: No boiling after the addition of millet flour	56.7%(n=17)
Method 2: Second boiling after the addition of millet flour	43.3%(n=13)

Table 1 above is a representation of the *thobwa* preparation process as reported by the 30 interviewed respondents and as observed in most households visited, summarised in two main processes.

4.3. Practices of food handlers during *thobwa* processing

To begin with, the respondents were asked about their source of water which is used in the *thobwa* preparation process. Figure 3 below is the presentation of findings, on practices as responded by the *thobwa* producers at household and selling point level.

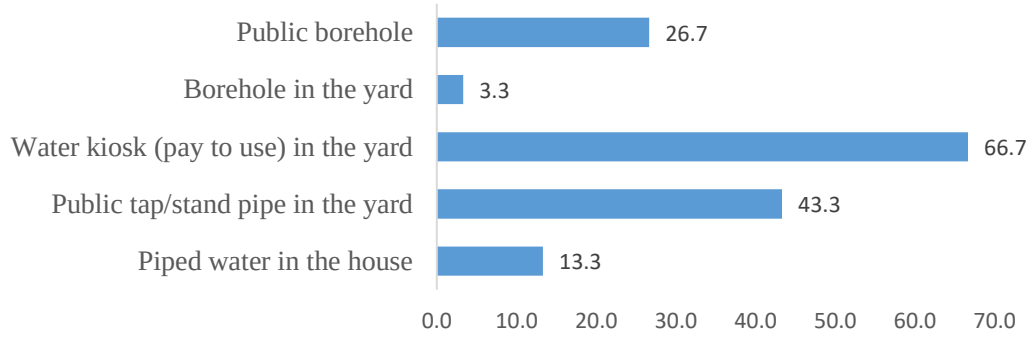

Figure 3: Water access for thobwa producers

Another area of focus was on trying to look at the treatment given to the water before it starts being used. The findings indicated that 86.7% (n=26) of the respondents were not treating their water before using it despite the source of collection.

In trying to look further on the hygienic practices, the study revealed that only 16.7% (n=5) of the respondents did not have a toilet to use at home. Of the 83.3% (n=25) who responded to having a toilet, 75% (n=18) use a pit and concrete slab toilet. Furthermore, of those who responded to have a toilet, 85.2% (n=21) reported that the toilet is shared among several households. Apart from that, the study looked at the availability of handwashing facilities close to the toilet in functional state. The findings indicated that 58.6% (n=18) of the respondents did not have a functional hand washing facility. In terms of handwashing behaviour, *figure 4* below is revealing recall on how often the respondents can wash their hands.

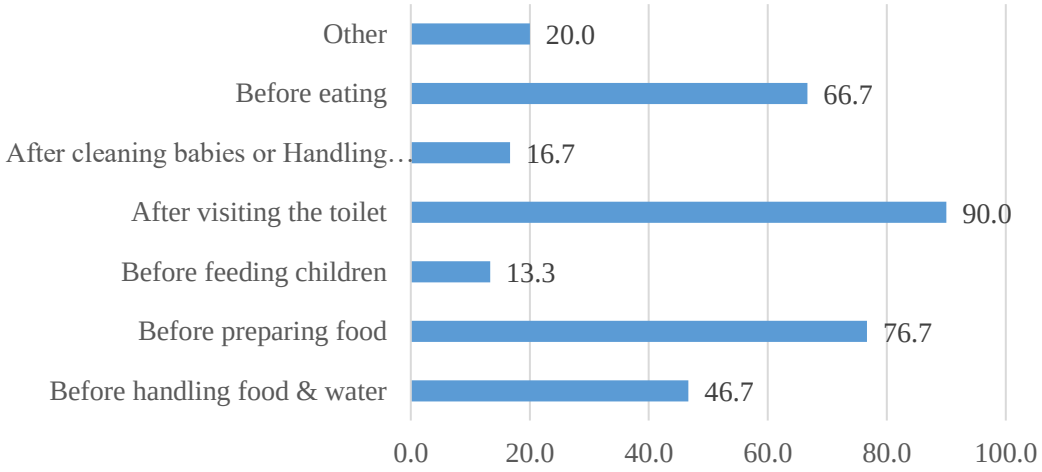

Figure 4: Handwashing frequency recall

On the issues of sanitation and toilet use, 83.3% (n=25) of the respondents reported to have a toilet facility which they use at home. Of those, most of them were also sharing a facility with so many households, at a percentage rate of 85.2 while 14.8% responded to having a toilet of their own.

The type of toilet, which was more common in most households around Ndirande, with much focus to the *thobwa* producers was a pit latrine with concrete slab and a locally smeared pit latrine, at a percentage of 60 (n=18) and 26.7 (n=9) respectively.

Another area worth mentioning is the availability of drop-hole cover in most toilets. According to the study findings, 56.7% (n=17) of the toilets had drop hole covers while 43.3% (n=13) of the toilets visited did not have a drop hole cover.

Apart from the issues of sanitation, waste management was checked as presented in the *Table 2* below.

Table 2: Waste disposal, pets, and microorganisms' prevention

Household wastes disposal		Frequency (N=30)	Percent (%)
	Garbage pit	20	66.7
	Tightly closed Bin & plastic containers	4	13.3
	River and offsite open dumping	6	20
Type of pets accessing the kitchen		(N=30)	
	Cats	1	3.3
	Dogs	4	13.3
	Neighbours' Dogs & Chickens	3	10
	Secure kitchen with no access to pets	22	73.3
Thobwa prevention mechanism		(N=30)	

	Cover with tight lids	22	73.3
	Cleaning containers	21	70.0
	Cooking inside the HH	17	56.7
	Cooling in fridge	13	43.3
	Reheating the Thobwa	10	33.3

In addition to waste management, the study further looked at the management of pets like dogs in relation to their access to the kitchen where food is prepared. On this, the study found that 26.7% (n=8) of the respondents had their kitchen accessed by dogs of their own or from neighbours. The study further looked at the prevention mechanisms of the *thobwa* from microorganism during preparation. Of the multiple responses received, 22 respondents mentioned covering the *thobwa* with tight lids, seconded by 21 who mentioned cleaning the containers as an option. On a lower side was 10 of the 30 respondents who mentioned reheating the *thobwa* as an option for prevention of microorganisms which would contaminate the *thobwa*.

4.4. Levels of contamination in the locally brewed *thobwa*.

From the key findings of the laboratory investigations, out of the 37 *thobwa* samples collected, the table 3 below is explaining the behaviour of the *thobwa* samples.

Table 3: Behaviour of collected thobwa samples

Process of preparation	**Percentage**
Method 1. No boiling after the addition of millet flour	8% (n=3)
Method 2. Second boiling after the addition of millet flour	92% (n=34)

Out of these samples in the table above, when combined, 21 were found with *E. coli* while 25 and 28 samples were found with *Salmonella* and *Staphylococcus* respectively despite being boiled for the second time or not.

In terms of percentages, the table 4 below is giving an overall analysis of contamination findings in general.

Table 4: Contamination levels of thobwa

Type of organism	**Percentage**	**CFU ranges**
E. coli	56.7 (n=21)	$1.0x10^6$-$9x10^4$
Salmonella	67 (n=25)	$1x10^4$-$3x10^6$
Staphylococcus	75 (n=28)	$1.6x10^8$-$2x10^4$

This then means that 56.7% of the total samples collected had all the three microorganisms under investigation while in terms of levels of contamination, *Staphylococcus* seem to be more present in most samples collected.

Further analysis was conducted to appreciate the levels of contamination of the *thobwa* and of the bottles at different stages of preparation before it reaches the final user who is the consumer at the market. The outcome of both *E. coli*, *Salmonella* and *Staphylococcus* contamination during the first and second incubation are presented on the *Table 5* below.

Table 5: Stages of contamination in thobwa preparation

Sample	**Total Samples**	**Frequency (%)**	**Type of contaminant (number: Range of CFU/100mls)**
Last cooking point *thobwa*	37	19 (63)	E. Coli 18: ($1x10^6$-$3x10^4$) Salmonella 15: ($1.2x10^4$ -$3x10^6$) Staphylococcus 19: ($1x10^6$ -$2x10^4$)
Last cooling point *thobwa*	37	23 (77)	E. Coli 18: ($1.3x10^4$-$6x10^4$) Salmonella 20: ($1.5x10^6$ -$3x10^4$) Staphylococcus 21: ($1.1x10^4$-$2x10^6$)
Selling Point *thobwa*	37	32 (86)	E. Coli 30: ($1x10^6$-$9x10^4$) Salmonella 15: ($1.2x10^4$ -$3x10^6$) Staphylococcus 28: $1.4x10^6$ - $2x10^8$
Used bottle swabs	14	8 (57)	3-172MPN/100mls
Hand swabs	14	8 (57)	1-2419MPN/100mls
Empty bottle swab	14	5 (36)	1-7MPN /100mls
Water	23	15(70)	1733-2419MPN/100mls

Looking at the Table 5 above, the results indicate that there was contamination in both the *thobwa* itself, bottles used in packaging and even the hands of food handlers, handling the *thobwa*. In addition to the samples mentioned, 28 water samples were also collected and only 23 were analysed, representing 88.46% of samples. From the analysed samples, the outcomes are outlined in Figure 5 below.

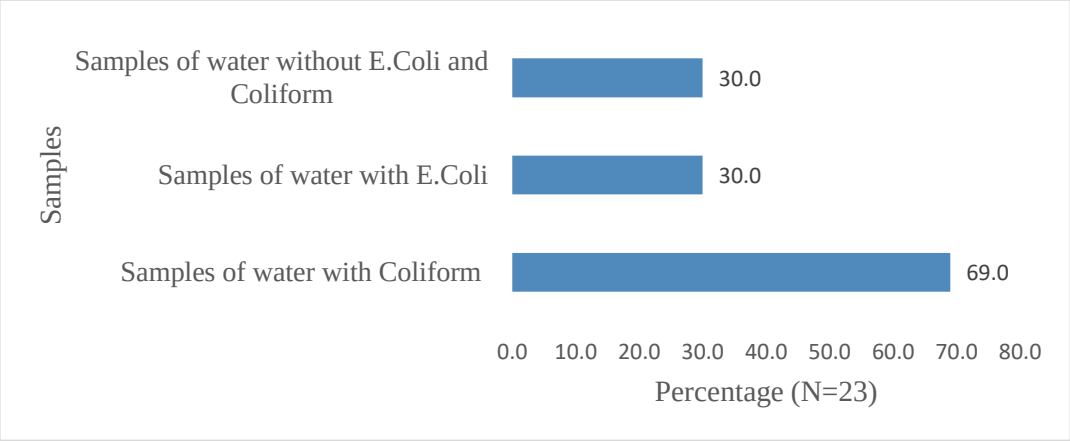

Figure 5: Water contamination levels

4.5. Points of contamination for the *thobwa*

Points of contamination are areas where contamination takes place more if compared to other points in the *thobwa* preparation. Steps were observed during the preparation until when the *thobwa* was being sold to the customers. Furthermore, water tests were also done for the water which was used during the preparation stage. Water tests were necessary as it was indicated that only 13% (n=5) of the respondents were able to treat their water with chlorine before use. Of the water samples collected, 47.8% (n=12) were collected at the selling point, labelled as used water while the other percentage was collected at home, labelled as water before use. The laboratory findings of the tests of water samples are explained in *Figure 6* below:

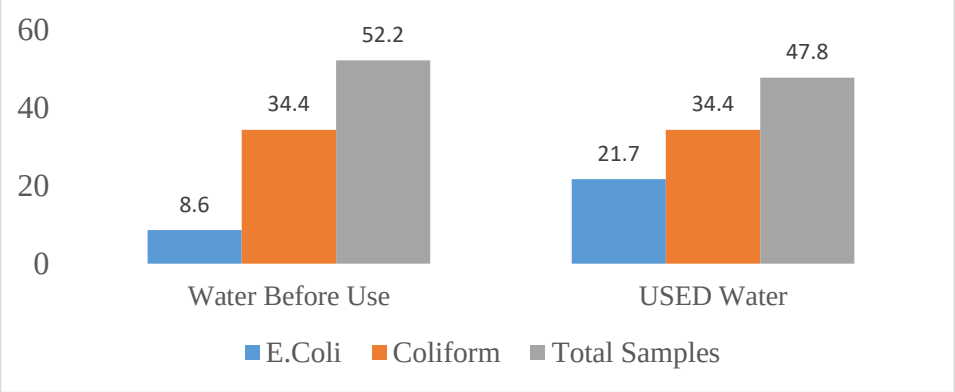

Figure 6: Contamination levels of water at home and market

In addition to the above, the overall outcome was that 30% (n=7) of the total water samples collected and found with *coliforms* had growth of *E. coli.*

Apart from the water tests, bottle swabs as well as hand swabs were also taken in all the 14 respondents whose *thobwa* samples were also taken for testing. Notable in the results were increased levels of contamination in used bottles with positive *coliform* and *E. coli* of 61.5% (n=8).

4.6. Development of a HACCP system to reduce *thobwa* contamination

The study further tried to develop a HACCP system to use in controlling the contamination levels in *thobwa* production process. To achieve that, it first looked at the critical control points in the entire process of *thobwa* production before it reaches the end user who is the consumer, by looking at the relationships of common behaviours of the food handlers who prepare the *thobwa* against the levels of contamination in different stages of *thobwa* preparation. The preparation process of the *thobwa* based on the study findings identified raw materials used in the *thobwa* preparation, the hygienic standards of the food handlers and the actual process of *thobwa* preparation. The results which were queried in SPSS for Windows 20, to identify such kinds of relationships under HACCP principles are presented in different tables, summarised below.

Table 6: Comparing association of pets with thobwa contamination

Independent variable		If the pets have access to the kitchen where *thobwa* is prepared				
		No	**Yes**	**Chi-Value**	**P-value (0.05 sign level)**	**Sample size (n=14)**
Last cooking point of *thobwa* at home	No	5	1	1.66	0.198	14
	Staphylococcus	4	4			
Selling point of *thobwa* in bottles at the market	**No**	**0**	**1**	**4.2**	**0.04**	**14**
	Staphylococcus	**9**	**4**			
Last cooling point of *thobwa* at home	No	2	3	1.99	0.158	14
	Salmonella	7	2			
Selling Point of *thobwa* in bottles at the market	No	1	3	1.59	0.207	14
	E-coli	8	2			

The Table 6 above presents the findings on *thobwa* contamination in association with the presence of pets and their access to the kitchen. Of particular interest to the findings was a relationship which was found that having pets access to the kitchen has a statistically significant association (p-value: 0.04) with staphylococcal contamination in the *thobwa*. This then means that having pets which access the kitchen where *thobwa* is cooked is 4.2 times more risk of having contaminated *thobwa* with Staphylococcus at the selling point.

Another area of interest was that of water used for cooking *thobwa* at household level. The comparison is narrated in the Table 7 below.

Table 7: Comparing association of contaminated water with contamination of thobwa

Independent variable		Water at home used for cooking thobwa				
		No	Contaminated	Chi-Value	P-value (0.05 sign level)	Sample size (n=12)
Last cooling point of *thobwa* before bottling	No	5	1	4.7	0.03	14
	E-coli	2	6			
Last cooling point of *thobwa* before bottling	No	3	2	0.31	0.577	14
	Salmonella	4	5			
Last cooling point of *thobwa* before bottling	No	4	0	5.6	0.018	14
	Staphylococcus	3	7			

The findings in the table 7 above, compared the association between contaminated water which was used to cook *thobwa* with the contamination of *thobwa* by different bacteria after being left to cool. It was found that the association between the presence of *E. coli* and *Staphylococcus* in *thobwa* at the last cooling point were statistically significant to contaminated water used for cooking *thobwa*. This is the case as there was 4.7 (p-value: 0.03) times risk of getting *E. coli* in *thobwa* at the last cooling point if the water used to cook *thobwa* was contaminated. Likewise, there was 5.6 (p-value: 0.018) times risk of getting S*taphylococcus* in *thobwa* at last cooling points if the water used to cook the *thobwa* was contaminated.

The correlation was also conducted to appreciate the contamination levels of *thobwa* versus the time when the sample was collected. Figure 7 below is showing the results.

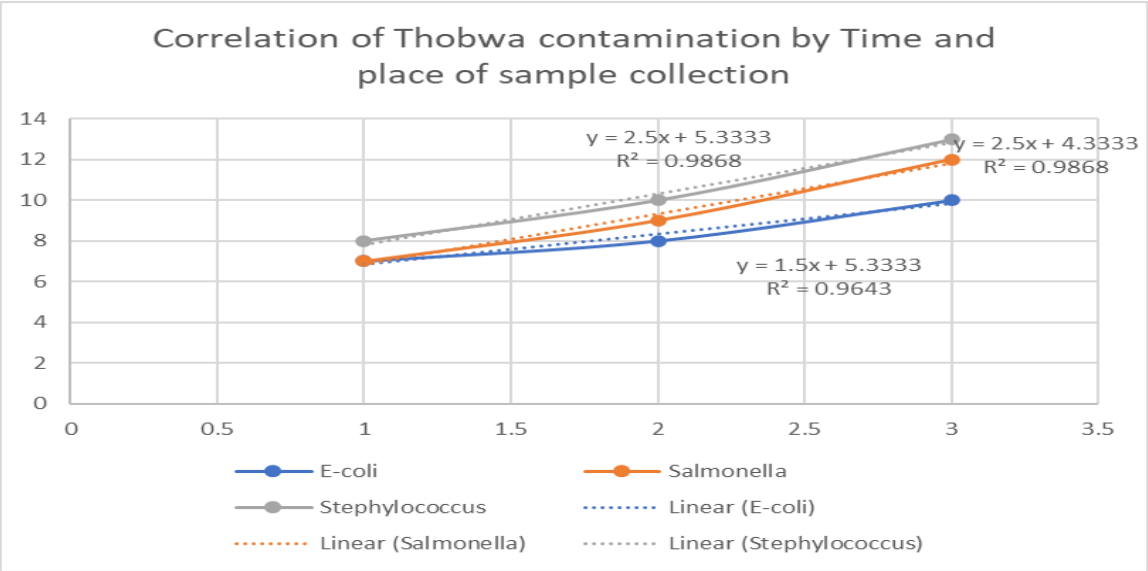

Figure 7: Correlation of thobwa contamination by time of collection

From the figure 7 above, there was a positive correlation in all the three types of contamination against the time when the sample was collected. Stage 1 was same day when the *thobwa* was cooked at home in the evening when *thobwa* has just been cooked, while stage 2, was the following day in the morning, at the same household, before the *thobwa* was bottled while stage 3 was at the selling point, when the same *thobwa* was in the bottle, collected at least in the afternoon. Therefore, this analysis indicates that, the increase of the stages of *thobwa* processing will also increase the chances of contamination of *thobwa*. However, all these correlations were not statistically significant, as the following p-values were 0.124, 0.073 and 0.073 for *E-coli Salmonella and Staphylococcus* respectively.

Again, all these findings must be interpreted with caution because the sample size was very small to generalise any specific relationship in the *thobwa* contamination.

Having noted the *thobwa* preparation process and the relationships with different stages and behaviours of food handlers, versus levels of contamination, the first level of *thobwa* preparation as found in Ndirande-Makata area, based on the findings of the study is ending at when the maize porridge has been mixed with millet flour to come up with the so called *thobwa*. The subsequent stage in the preparation of the *thobwa* included the cooling stage when the *thobwa* is left to cool for a night, and the third stage is when there is use of bottles in packaging the *thobwa* before selling process. The packaging was also done by hands of food handlers. The study has revealed the increased levels of contamination for the hands of food handlers and bottles which are used for packaging the *thobwa*, even though the association is not statistically significant. To this effect, there are several steps to follow to make sure the *thobwa* is free from

microorganisms. All the identified critical control points found during the process of the study are summarised with their critical limits and control measures in *Figure 8* below.

HACCP ANALYSIS FOR THOBWA PRODUCTION

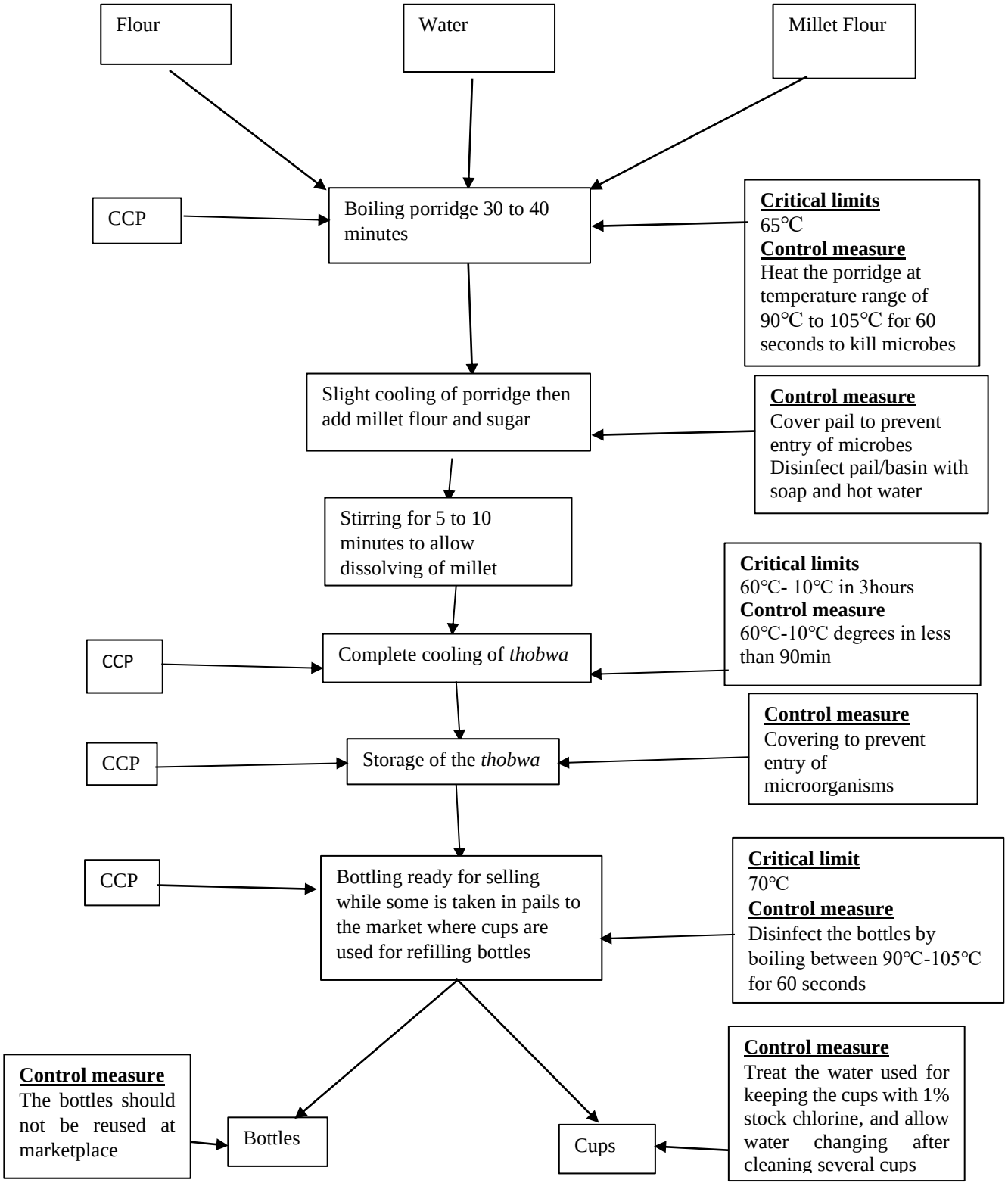

Figure 8: HACCP complete analysis for thobwa production

There are four critical control points which may need to be investigated as narrated in the Figure 8 above. These areas include the boiling point stage, cooling stage, storage and the bottling stage which includes handling of the *thobwa* at market level. Critical limits and control measures are well narrated in the same figure above, to make sure the product reaches the end user in a good and safe state.

CHAPTER FIVE

DISCUSSION

5.1. Introduction

This chapter is discussing the results of the study, the difference and similarity with other similar studies conducted by others in a slightly similar topic, the understanding and interpretation of the researcher based on the results findings.

5.2. Demographic findings of the respondents

The majority of *thobwa* sellers were women and these women also turned out to be household heads and bread winners as evidenced by a small percentage of the respondents who were married and supported by their husbands. This is also in line with several studies which indicate that women are the most hit by economic factors and they resort into small scale businesses for family survival as in the Malawi Demographic Health Survey of 2015 National Statistical Office (NSO,2017). Furthermore, the study also revealed that *thobwa* business is commonly done by people whose education levels are low as the study results revealed that the majority of the *thobwa* sellers are primary school dropouts.

The education level revelation is interesting as it had also gone in line with the type of activity which the respondents are able to do as part of their earning a living apart from *thobwa* selling. The study did not find anything new as it found that more than half of the respondents depend on the *thobwa* business to earn a living, a small-scale business, in line with the findings of Malawi Demographic and Health Survey, a nationally covered study by government which also found that small businesses are survival means for low-income earners in both rural and urban Malawi (NSO, 2017)

5.3. Hygienic practices of food handlers

The analysis revealed that, most of the study respondents have easy access to safe drinking water despite the distance, a situation which is similar to other studies done like the (UNICEF, 2018) statistics which indicates that around 87% of urban dwellers have access to basic drinking water, while at national level the percentage is at 67. What is silent from the statistics is the percentage of those who are drinking safe water, as the study revealed that those who had drinking water were covering a certain distance to collect the water and they were not able to cover their bucket with a lid, thereby exposing the water to contamination with microorganisms (UNICEF, 2018). This study also found that access to safe drinking water does not guarantee

the actual safety of the water as the analysis further revealed that, despite the longer distance covered by women to collect their water from a water kiosk, only 10% (n=3) were able to treat their drinking water with chlorine. Nearly 90% of the study respondents were using their water without being treated despite environmental exposure. This is also in line with another study conducted in 2016, in the neighbouring Zambia by Rosa et al., (2016), which found that nearly half of urban dwellers were drinking treated water at point of use, meaning there is also a higher percentage of urban dwellers who were not treating their water at point of use despite the distance covered on water collection (Rosa et al., 2016). In a previous study done locally, almost ten years earlier than the Zambian study, Lauren Stockman, and others, also found that only a very small proportion of their study respondents were using point of use water treatment methods as a water treatment measure, despite the distance covered to collect the water (Stockman et al., 2007). This is telling us that at the local level, the trends of point of use water treatment have not changed much if the findings of this study are something to go by. On a different note, this study found that more than half of the water samples tested were contaminated with environmental coliforms. According to (Mahon, 2007), it is indicated that if coliform bacteria are present in drinking water, there is an increased risk of contracting a water-borne illness, when using that water. There is an increased risk of diseases on the bottled *thobwa,* as the water found with coliforms is the same water which is used to cook the *thobwa* and clean up the bottles used for packaging the *thobwa*. Although total coliforms can come from other sources than faecal matter, a positive total coliform sample should be considered an indication of pollution in your water. Positive faecal coliform results, especially positive *E. coli* results, should be considered indication of faecal pollution in the water (Mahon, 2007). This is in total agreement with the findings of this study which established some relationship of water contamination with the risk of having *Staphylococcus and E. coli* at last point of cooling *thobwa* and at the selling point too if the water used is not treated at home after collection, and before the water is used. Furthermore, when the study looked at the hygienic practices of the food handlers including handwashing, it was revealed that handwashing before feeding children and handwashing after cleaning babies or handling children's stools was mentioned by a small percentage of respondents. This might be that a small percentage of respondents had small babies making that parameter not applicable to most of the respondents. However, it may also mean that most respondents, who were also food handlers, turn to forget washing their hands after handling baby stools. The hand swabs taken to check contamination levels of the hands of *thobwa* producers revealed that more than half of the *thobwa* producers had contamination of either *E. coli*, *Salmonella* or *Staphylococcus*. This again, simply means that, all the hands that are not washed with soap and water after handling baby stools are at great risk of being

contaminated with *E. coli*. This same microorganism can easily be transmitted from the hands to the food which the handlers are preparing and or selling. Even though this study did not find any correlation of handwashing with results of the hand swabs, the increased levels of contamination of the *thobwa* can as well be attributed to the contamination of the hands as evidenced by previous studies conducted by (Chidziwisano et al., 2019) where they found that a very small percentage of the respondents were able to wash their hands with soap before food preparation and before feeding a child. This study findings could not find the association, maybe because the sample size was very small to establish something significant but, the study found an increase of *thobwa* contamination as the stages are increasing in preparation even though not statistically significant but, something which cannot just be ignored. These findings are also not different to the findings of another study which was conducted in Mzuzu-Malawi, where water contamination levels at fresh fish markets were also being assessed (Lazaro et al., 2019). This then means that water use at market level is not a guarantee for hygiene and sanitation practise as the water being used might have high levels of contamination thereby compromising the practised behaviour.

Apart from the handwashing and water treatment, the study also looked at the toilet use of the respondents. What was found indicates that most of the respondents were sharing their toilets with their neighbours, a behaviour which compromised the sanitation of the toilet, thereby increasing the risk of faecal contamination to the water and food including *thobwa* which was the focus of the study. This study failed to establish any significant relationship on those *thobwa* producers with the availability of drop hole cover of the toilet, maybe because of the small level of sample collected. In terms of hygiene practices of the food handlers, the study with specific reference to other studies done by others, has revealed the gaps in the levels of hygiene and sanitation which may have an attribute towards the quality of the *thobwa* being prepared for local selling and consumption of industrial workers.

5.4. *Thobwa* contamination levels

The study findings also revealed that food handling has a great influence in the contamination levels of the *thobwa*. The good example is the increased level of contamination of at least more than 10%, which was noticed throughout the stages of preparation, and the analysis of this study, found some correlation which was not statistically significant on increase in the contamination levels as the preparation stages were advancing. The most important issue worth discussing is the *thobwa* preparation stages itself. There were almost half of the respondents of the questionnaire that reported that they do not re-boil their *thobwa* for the second time before bottling is done, meaning the millet flour made from germinated seed was not fully cooked and

yet the *thobwa* is ready for selling at the market. When samples were collected, only three respondents indicated not to have to re-boil the *thobwa* for the second time, meaning most of the samples collected were re-cooked after the addition of millet flour yet the *thobwa* was still found with contamination of *E. coli* and *Staphylococcus*. The contamination was more eminent with use of contaminated water, even after the *thobwa* had been cooked and left to cool, meaning the cooking itself wasn't enough to kill the microorganisms in the water and that *E. coli* dominated the contamination levels. In another study related to food preparation and hygiene, (Chidziwisano et al., 2019) found that there are also great opportunities for cross contamination due to lack of handwashing and multitasking during cooking like the ability of the mother to remove child's mucous and then resume cooking even before hand washing is done (Chidziwitsano, et al., 2019). Further studies may need to be done with a larger sample, to appreciate the contribution of uncooked millet flour from germinated seed, and the handling process of food handlers to the contamination levels of the *thobwa*. On the part of contamination, the study further revealed that slightly above half of the *thobwa* samples analysed had growth of both *E. coli, Salmonella* and *Staphylococcus*. In terms of CFUs, the Malawi Bureau of Standards (MBS, 2016) recommendation for *thobwa* is zero presence of *Salmonella* and *E. coli* while *Staphylococcus* must be less than 10^2, a situation which is very different with the findings of this study Malawi Bureau of Standards (MBS, 2016). This then means that more than half the total samples tested were contaminated besides being prepared for human consumption. The contamination might be due to the water used, hands and other handling techniques of handlers, as the study also revealed the level of contamination of the hands which were handling the *thobwa* during preparation. Again, this study failed to establish any association between the levels of contamination and the hygienic behaviours of the food handlers, except the availability of pets which access the kitchen, which was established to have a statistically significant association with *thobwa* contamination, especially that *thobwa* sourced from the selling point level. In terms of levels of contamination, *Staphylococcus* was more present in most samples collected at either during the first or second incubation. As already discussed above, the presence of *Staphylococcus* is something which may need to be looked at with caution as this may look harmless, yet it can simply cause food poisoning with the toxins from the bacteria. According to (Mahon, 2007), the common way of getting *Staphylococcus* in the food is through food handlers who carry the bacteria in their body and careless unhygienic food handling which might have caused contamination to the food (Mahon, 2007). But this study did not establish any statistical relationship of the food handlers, in the way their practice contributes to *thobwa* contamination.

5.5. Critical Control Points (CCP)

The study also looked at critical control points to establish the contamination of the *thobwa* which is consumed by low-income earners in the industrial area. The study found that the contamination was increasing as the *thobwa* was staying longer after being cooked due to environmental situation of the storage area and maybe the pets, as the *thobwa* is left uncovered for a night to facilitate complete cooling, poor hygiene practice by food handlers increases the risk of contamination for *thobwa* as noted by the level of contamination of the hands of food handlers and bottles used in packaging the *thobwa*. As already stated, this study did not find any statistical correlation in most relationships analysed but it failed to reject the relationship between levels of contamination in association with pets' access to the *thobwa* in the kitchen, meaning there is direct positive correlation of pets accessing the kitchen with levels of *thobwa* contamination. These findings concur with Chidziwisano et al., (2019) who found a similar issue of poor hygiene practises, food storage and inadequate pre-consumption heating as contributors for high risk of food contamination at household level (Chidziwisano et al., 2019). The critical control points according to the findings of this study includes the boiling of the *thobwa* at the very first stage of preparation, starring and cooling stage after the *thobwa* porridge has been mixed with Millet flour from germinated seed and the packaging of the *thobwa* into the bottles, in readiness for selling. In addition, the study also identified the unwashed hands as potential for contamination for the *thobwa*, which is also in line with another study conducted by Malangu (2016), which found that the unclean hands have a potential of contaminating food during preparation or handling after preparation. According to Malangu, street food is frequently cooked well in advance of consumption, but it is prone to contamination from exposure to dust, pets, flies, and the handling of the food handlers. The handling and processing of food by the handlers who may be carrying germs open opportunities for contamination of food if adequate precautions are not implemented (Malangu, 2016). This is also in line with the study findings as it found that the access of the pets to the kitchen where food is prepared has a direct relationship with contamination levels of *thobwa*, with specific focus to *Staphylococcus*. This study findings, further identified hands as the source of contamination for the *thobwa* sellers, though not statistically significant. This is again in line with the food guidance report of 2006 which indicated that contaminated equipment and poor personal hygiene contributes to the contamination of the food (Center for Food Safety and Applied Nutrition, 2006). Most open food outlets have a biggest problem on contaminated equipment and poor personal hygiene as being the highest risk factors, something which even this study also found, as the bottles and water used in cleaning the bottles and cups including drinking were found with levels of

contamination. This is also in line with a study in Turkey, where they found that food handling leaves a lot to be desired as far as handling and contamination are concerned, as they revealed the relationship of food preparation in kitchen and those without a kitchen, versus the contamination levels of food (Takanashi et al., 2009). This is also in line with the study findings which found that 73% of the respondents had a secure kitchen which animals could not have access to it, but the small section whose kitchen was not secure and had direct access to entry, by pets, which had a direct significant association with the positive results of *Staphylococcus* in the *thobwa* samples at selling point. This then means that for the safety of the food to be maintained, critical areas including control of pets' access to the kitchen may need to be looked further and controlled to eliminate contamination before the end user accesses the *thobwa*.

In summary, there are four critical control points which may need to be investigated. These areas include the boiling point stage, cooling point stage, the bottling stage, and the handling stage at market level. Apart from that, the water which is used in the *thobwa* preparation stages, and the bottles used in packaging the *thobwa* may need to be taken care of if contamination must be eliminated completely, despite some results not being statistically significant due to the population size of the study. Measures include but not limited to following principles of general hygiene and sanitation at household level, proper handwashing with soap at critical times, more especially when in the process of *thobwa* preparation in all the stages, cleaning of utensils which are used during the entire *thobwa* production process, water treatment with specific focus to the drinking water and that water used to clean utensils and also the avoidance of recycling the bottles on the same day when *thobwa* is being sold at the market. Furthermore, the critical limits and control measures need to be taken care of, to eliminate contamination in critical points of *thobwa* preparation.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The contamination levels of the *thobwa* itself and the bottles used for packaging the *thobwa* is something which cannot be overlooked. The study has established contamination of *thobwa* which is sold to the people. The study has also established the behaviour and hygiene practises done by the food handlers in the home, especially during the preparation of the *thobwa*. Furthermore, the study has also established the increased levels of contamination of the *thobwa* as the stages in preparation are advancing. Even though the findings indicate some statistical associations which are not significant and some statistically significant findings, the sample size was very small to generalise any specific relationship in the *thobwa* contamination. Following the HACCP principles, it has also been revealed in the study that there are specific areas and specific points when contamination can take place during the preparation of *thobwa*. That is to say even though this is the first study of its kind in Malawi, covering a very small section of Blantyre city with a very small sample size, it has still ended up in proving the presence of contamination for the *thobwa* drink sold by vendors, whose hygienic and sanitation practices during preparation have a certain compromise as established in the study.

6.2. Recommendations

The study recommends:

- That *thobwa* sellers may have to be oriented by the city assembly department of public health, in the better preparation of the drink to reduce risk of contamination to the end users.
- The involvement of city assembly for mass communications to all venders around Blantyre city, with specific focus to food sellers on hygienic standards and importance of handwashing during food handling.
- The intensification of hygiene and sanitation messages to all community members, with specific focus to those in urban or peri-urban setup especially the food selling handlers.
- That the interventions to reduce contamination risks should focus on critical control points in *thobwa* preparation, storage and reheating of the *thobwa* and other pre-selling contaminations like food handlers' hygiene and sanitation and bottles used for packaging the *thobwa*. Adequate pre-packaging heating of the *thobwa* after the addition of millet flour, improvements of proper hygienic practises in the *thobwa*

preparation, elimination of pets' access to the kitchen, cleaning of utensils, hands and use of safe and protected water when washing the hands and cleaning the utensils may help in reducing the contamination in all the critical points.

- A bigger study to ascertain contamination levels in the *thobwa* and other related food items sold by venders in the cities as the risk of contamination is very high.

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APPENDICES

Appendix 1: Household level individual respondent questionnaire

Individual Respondent Questionnaire for Thobwa Producers and Sellers

A. INTRODUCTION				
My name is Phillimon Phiri, a student of the Polytechnic conducting an academic research on food hygiene, specifically for Thobwa. I would therefore like to interact or discuss and learn from you on how issues of water, sanitation and hygiene and food preparation are handled here. No names will be mentioned as we are only interested on ideas for academic purpose. So feel free to respond and ask questions where possible. The interview may take 20minutes. Can we proceed?				
		Yes		continue to next section
		No		end the interview
B. DEMOGRAPHICS				
	Question	**Response**	**Codes**	**Logic**
B1	**Respondent ID Number**			
B2	Respondent Sex	Male	1	
		Female	2	
B3	Respondent Age			
B4	What is your position in the household	Male head of HH	1	
		Female head of HH	2	
		Wife of the head of HH	3	
		Son of head of HH	4	
		Daughter of head of HH	5	
		Mother of head of HH	6	
		Father of head of HH	7	
		Other female	8	
		Other male	9	
		grandmother	10	
B5	Highest level of education	No education	0	
		Primary education	1	

		Secondary education	2	
		Tertiary education	3	
B6	If never been to school, do you know how to read and write	No	0	
		Yes	1	
B7	Apart from this business, what else do you do for a living? (Choose all that apply)	None	0	
		Farming	1	
		Self-employed/skilled worker	2	
		Salaried government	3	
		Salaried private sector	4	
		Other (specify)	5	
C. HYGIENE				
	Question	**Response**	**Codes**	**Logic**
C1	Where do you access your water for producing thobwa?	Piped water in the house	1	***(Multiple response)***
		Public tap/stand pipe in the yard	2	
		Water kiosk (pay to use) in the yard	3	
		Borehole in the yard	4	
		Public borehole	5	
		well	6	
		Surface water (River, dam, stream)	7	
		Other (specify)	8	
C2	How is the collected water stored before use?	Plastic containers with a lid	1	***(Multiple response)***
		Plastic containers without a lid	2	
		Clay containers with a lid	3	
		Clay containers without a lid	4	
		metal pail(ndowa)	5	
		metal drum	6	
		jerrycuns (5,10 or 20litres)	7	

		small bottles	8	
		plastic drums	9	
		other (specify)	10	
C3	Is there any treatment done to the water before you start using, after collection?	No	0	***if No>> to C5***
		Yes	1	
C4	What sort of treatment does the water have before use? (for surface water and wells) **(*Select multiple answers)***	None	0	
		Boiling	1	
		Add chlorine/water guard	2	
		Strain it through a cloth	3	
		Let it stand and settle	4	
		Other (specify)	5	
		Don't Know	99	
C5	Do you clean the kitchen utensils including bottles and cups using the water mentioned above?	No	0	
		Yes	1	
C6	How do you store your kitchen utensils	in a covered bucket	1	
		In a closed cup board	2	
		in an open bucket	3	
		on an open space	4	
		Other (specify)	5	
C7	What happens to the utensils after use	raid on the floor	1	***if 1>> to C11***
		placed in a basic for washing later	2	
		They are disposable	3	
		Other (specify)	4	
C8	How are the utensils cleaned?	water only	1	
		water with soap	2	
		water with sand	3	
		Flour	4	
		others (specify)	5	
C9	How do you dry your utensils	dish towels	1	
		Air drying	2	

		never dries	3	
		others (specify)	4	
C10	Do you have pets with you at this household	No	0	
		yes	1	***if Yes>>mention them***
	Pets name list			
C11	Is there anything you do to prevent insects and other related rodents from accessing the kitchen utensils and food in the kitchen	No	0	***if No>> D1***
		Yes	1	
C12	What is it that you do to prevent rodents and other insects from accessing the food and utensils	the kitchen is always closed and monitored	1	
		cover the food items	2	
		pets always in closed doors away from kitchen	3	
		utensils always in cupboard	4	
		Other (specify)	5	
D. SANITATION				
	Question	**Response**		**Logic**
D1	Do you have a sanitary facility on the premise which you can use?	No	0	
		Yes	1	***If 1>> to D3***
D2	Where do you urinate/defecate?	Public toilet	1	
		neighbour	2	
		plastic bag	3	
		river	4	
		bush	5	
		other(specify)	6	
D3	How many sanitary facilities do you have for the household?			
D4	What type of sanitary facility (s) do you have?	flush toilet & septic tank	1	
		pour flush toilet & septic tank	2	
		pour flush and pit	3	
		pour flush with concrete slab	4	

		concrete slab & pit	5	
		wood board & pit	6	
		Urine diverting toilet	7	
		locally smeared latrines	8	
		other (specify)	9	
D5	Does your sanitary facility have a drop hole cover	No	0	
		yes	1	
D6	How many household members use the sanitary facility?			***(numeric)***
D7	Is the sanitary facility shared among several households?	No	0	***If No>> to D10***
		Yes	1	
D8	How many other households?			
D9	How many neighbors and other people in total use the sanitary facility?			
D10	Do you have a handwashing facility (ies) which you use?	No	0	
		yes	1	***(If yes observe)***
D11	How often do you wash your hands	before handling food & water	1	***(More than one option possible)***
		before preparing food	2	
		Before feeding children	3	
		After visiting the toilet	4	
		After cleaning babies or handling children stools	5	
		Before eating	6	
		Other (specify)	7	
D12	What do you use when washing hands?	water only	1	
		water and ash	2	
		water and soap	3	
		other(specify)	4	
D13	Why do you wash your hands	no idea	0	
		to prevent contamination	1	
		to prevent disease	2	

		to be clean and remove dirt	3	
		others (specify)	4	
D14	Do you think people can get sick from eating/drinking thobwa?	No	0	***(If no >> to D16)***
		Yes	1	
D15	Why should people get sick after drinking Thobwa?			***After response >> to D17***
D16	Why should people not get sick when they drink thobwa?			
D17	Do you sell the thobwa yourself or someone else does that for you?	Self	1	
		someone does	2	
		both(self and someone)	3	
D18	How do you prepare the thobwa to ensure people do not become sick?			
E. ENVIRONMENTAL MANAGEMENT				
	Question	**Response**		**Logic**
E1	Where do you keep/store or dispose your household waste?	dumping in garbage pit	1	
		dumping in pit latrine	2	
		burning	3	
		dumping offsite	4	
		other (specify)	5	
E2	How do you dispose the waste?	containers (plastic, metal)	1	
		plastic bags	2	
		Dispose directly/no storage	3	
		other (specify)	4	
E3	Are there any recycling or re-using practices for managing the HH waste?	No	0	
		Yes	1	
E4	Do you have pets like dogs and cats at this household or from neighbors	No	0	
		Yes	1	

E5	Is your kitchen accessible by the Pets/Animals	No	0	***If No >> to E6***
		Yes	1	
E6	What pets/animals do have access to the kitchen? List them all			
E7	How do you prevent your food/thobwa from microorganisms	Nothing	0	***(More than one option possible, Interviewer probe to get more responses if available)***
		covering with tight lids	1	
		cleaning containers	2	
		Cooking is done inside the house	3	
		cooling in fridge	4	
		reheating the food	5	
		Other (specify)	6	
		Don't Know	99	

Appendix 2: Observational checklist at household level

HOUSE HOLD LEVEL OBSERVATION CHECKLIST

FOR THOBWA PRODUCERS HH LEVEL (25)

Zoyenera kuyang'ana pamalo pamene thobwa likuphikidwapo.

Checklist identification number: _____

Note: This checklist has sections and it has to be used at the household where thobwa is being prepared.

Mau otsogolera*: Ndondomeko zimenezi zikuyenera kuyang'anidwa pamene thobwa likuphikidwa.*

Section A1. Personal hygiene (*ukhondo wa pathupi***): *observe the look of the thobwa cooking person and check the following points (****yang'anitsitsani amene akuphika thobwa ndikuyankha mafunso otsatilawa:***

QA1.1	Wash hands after Toilet	Yes	No
QA1.2	Food handler to wash hands before handling food	Yes	No
QA1.3	Sneeze or cough near food	Yes	No
QA1.4	Do not smoke or eat in any kitchen areas	Yes	No
QA1.5	Tie back or cover long hair	Yes	No
QA1.6	Wear clean clothing	Yes	No
QA1.7	Wear an apron, clean catering t-shirt or catering jacket	Yes	No

QA2.1	Before handling food & water	Yes	No
QA2.2	Before preparing food	Yes	No
QA2.3	Before feeding children	Yes	No
QA2.4	After visiting the toilet	Yes	No
QA2.5	After cleaning babies or handling children stools	Yes	No
QA2.6	Before eating	Yes	No
QA2.7	Handling thobwa bottles	Yes	No
QA2.8	Packing the thobwa in bottles	Yes	No
QA2.9	Other	Yes	No

A2. Hand washing habits (take note of the handwashing habits focusing on when, how often, with what detergent and also check the critical times) *(kusamba mmanja)*

Other(Specify)……

Section B1:1 Environmental hygiene (*ukhondo pamalo ozungulira***): *observe the household surroundings and check if they have the following points. Check also if they use them by asking the respondent.* ***(****Yang'anani malo ozungulira panyumba ndikuyankha mafunso otsatirawa****).***

QB1. Where is thobwa prepared?

1. Inside the kitchen

2. Outside on veranda in yard

3. Outside on an open space in yard? etc.

Condition of the kitchen (hygienically good -this is a kitchen which has been either swept or mopped before use and utensils are not just scattered, no visible spider cobwebs etc.) *(ukhondo wamukhitchini)*

B1.1	Clean and Well-kept place	Yes	No
B1.2	Sheltered from dust, sun, rain and wind	Yes	No
B1.3	Far from all sources of contamination, solid waste	Yes	No
B1.4	Sweep the floor daily.	Yes	No
B1.5	Clean walls (Cobweb, Dirty, Visible dust)	Yes	No
B1.6	Clean up after each meal	Yes	No
B1.7	Clean spills when they occur	Yes	No
B1.8	Clean garbage disposal, Dust bin tightly closed	Yes	No
B1.9	Wash the floor weekly	Yes	No

Any other comments

……
……

QB2: Is there a handwashing facility available in the kitchen? How is the condition of the handwashing facility? *(Pali chosambira mmanja malo okonzerako chakudya?*

QB2:1	Availability of handwashing facility	Yes	No	Not applicable
QB2:2	Functionality handwashing facility	Yes	No	Not applicable
QB2:3	Water inside the hand washing facility	Yes	No	Not applicable
QB2:4	Can handwashing facility effectively be used	Yes	No	Not applicable

QB3. What about workbenches/counters, raw ingredient storage, evidence of rodents and insects, doors and windows open to the environment?

QB3.1	Store everything safely and securely	Yes	No
QB3.2	Knives should be carefully stored and carried	Yes	No
QB3.3	Cooking area floor safe	Yes	No
QB3.4	Adequate lighting and Ventilation in cooking areas	Yes	No
QB3.5	All equipment safely fixed/positioned	Yes	No
QB3.6	Wipe clean surfaces on all food preparation areas	Yes	No
QB3.7	Keep surfaces, chopping boards and utensils clean	Yes	No
QB3.8	Use double sink/bowls for washing and rinsing, using hot water and washing up liquid, for washing all crockery, cutlery and equipment	Yes	No

QB3.9	Ensure that adequate cloths/scorers are cleaned or replaced regularly	Yes	No
QB3.10	Dry everything in the air (or with disposable towels	Yes	No

QB4. Availability of animals (pets) like cats, dogs, chickens, etc. and their access to the kitchen or food preparation area.

QB4.1	Protect open food from flying insects	Yes	No	Not applicable
QB4.2	Check regularly for pests	Yes	No	Not applicable
QB4.3	Protect Thobwa from Cats	Yes	No	Not applicable
QB4.4	Protect Thobwa from Dogs	Yes	No	Not applicable
QB4.5	Protect Thobwa from Chickens	Yes	No	Not applicable
QB4.6	Protect Thobwa from Goats	Yes	No	Not applicable

Other……

QB5. Availability of drainage for wastewater and its hygienic condition *(Ngalande yotayira madzi-ikuoneka motani)*

QB5.1	Is the drainage system available	Yes	No	
QB5.2	Does the drainage system covered, (Use running water system properly)	Yes	No	Not applicable
QB5.3	Does the drainage system clean	Yes	No	Not applicable
QB5.4	Adequate and bins (covered) and bags (tied when full) for all waste	Yes	No	Not applicable

QB6. Type of Toilet/latrine usage (please check on the type of latrine and availability of drop hole cover, whether the toilet is shared or not. Is there anal cleansing materials inside the toilet?) *(chimbudzi chogwiritsa ntchito)*

QB6.1	Availability of toilet	Yes	No	
QB6.2	Flush Toilet & Septic Tank	Yes	No	Not applicable
QB6.3	Pour Flush Toilet & Septic Tank	Yes	No	Not applicable
QB6.4	Pour Flush And Pit	Yes	No	Not applicable
QB6.5	Pour Flush With Concrete Slab	Yes	No	Not applicable
QB6.6	Concrete Slab & Pit	Yes	No	Not applicable
QB6.7	Wood Board & Pit	Yes	No	Not applicable
QB6.8	Urine Diverting Toilet	Yes	No	Not applicable
QB6.9	Locally Smeared Latrines	Yes	No	Not applicable
QB6.10	Availability of Pit Latrines (Roofing)	Yes	No	Not applicable
QB6.11	Drop hole cover	Yes	No	Not applicable
QB6.12	Ventilation pipe	Yes	No	Not applicable
QB6.13	Faeces visible on the floor	Yes	No	Not applicable
QB6.14	Faeces visible on the walls	Yes	No	Not applicable

Other (Specify)……..

QB7. If the toilet is not available, where do they defecate? (*ngati chimbudzi palibe, mumakadzithandizira kuti?)*

QB7.1	Bush *(mutchire)*	Yes	No
QB7.2	River *(mumtsinje)*	Yes	No
QB7.3	Other household latrine *(kwa aneba)*	Yes	No

Others (specify)*(zina:fotokozani* …….

QB8. Is there a handwashing facility available, close to the toilet? How is the condition of the handwashing facility? *(Pali chosambira mmanja pafupi ndi chimbudzi?*

QB8.1	Availability handwashing facility	Yes	No	
QB8.2	Functionality handwashing facility	Yes	No	Not applicable
QB8.3	Water inside the hand washing facility	Yes	No	Not applicable
QB8.4	Can handwashing facility effectively be used	Yes	No	Not applicable
QB8.5	Availability of handwashing material (Soap/Ash)	Yes	No	Not applicable

QB9. Observe the general outlook and hygienic conditions of the containers used to keep thobwa preparation raw materials like maize flour, millet flour, including water source which is used for cooking thobwa. (*yang'anitsitsani ziwiya zomwe zikugwiritsidwa tchito pophika thobwa.)*

Conditions of the containers used to keep raw materials during Thobwa preparation

QB9.1	Maize flour/ Millet flour Storage Plastic Pail with a cover	Yes	No
QB9.2	Sugar Storage container with covers	Yes	No
QB9.3	Water Storage pail with covers	Yes	No
QB9.4	Other	Yes	No

Other Specify……

QB10. Observe the Source of water during food preparation

QB10.1	Standing water (Piped)	Yes	No
QB10.2	Surface water (lake, pond, basin, stream, river)	Yes	No
QB10.3	Ground water (Well, Borehole)	Yes	No
QB10.4	Other	Yes	No

Other Specify……

QB11. Observe the general parking of the final product and hygienic conditions of the bottles used to park thobwa ready for selling. (*yang'anitsitsani ziwiya zomwe zikugwiritsidwa tchito posugira thobwa lisanapite kumsika)*

QB11.1	Bottles bought from the shops	Yes	No
QB11.2	From a local supplier	Yes	No
QB11.3	Reused Bottles meant from other product	Yes	No
QB11.4	Cup bought from the shops	Yes	No
QB11.5	General cleanliness and disinfection of cups	Yes	No

General comments and other observations (*Ndemanga ndi zooneka zina pakhomo):*

……
……

End of observation checklist at household level

Appendix 3: Observation checklist at selling point level

OBSERVATION CHECKLIST

FOR THOBWA SELLERS AT SELLING POINT

Zoyenera kuyang'ana pamalo pamene thobwa likugulitsidwapo.

Checklist number: _____

Note: This checklist has sections and it has to be used at the selling point where thobwa is being sold.

Mau otsogolera*: Ndondomeko zimenezi zikuyenera kuyang'anidwa pamene thobwa likugulitsidwa.*

Section A:1 Personal hygiene (*ukhondo wa pathupi***): *observe the look of the thobwa seller and check the following points (****yang'anitsitsani amene akugulitsa thobwa ndikuyankha mafunso otsatilawa:***

QA1.1	Wash hands before and after handling food after Toilet	Yes	No	Not Observed
QA1.2	Customers to wash hands before eating	Yes	No	Not Observed
QA1.3	Sneeze or cough near food	Yes	No	Not Observed
QA1.4	Do not smoke or eat in any selling point areas	Yes	No	Not Observed
QA1.5	Tie back or cover long hair	Yes	No	Not Observed
QA1.6	Wear clean clothing	Yes	No	Not Observed
QA1.7	Wear an apron, clean catering t-shirt or catering jacket	Yes	No	Not Observed

QA2 Hand washing habits (take note of the handwashing habits focusing on when, how often, with what detergent and also check the critical times) *(kusamba mmanja)*

QA2.1	Before handling food & water	Yes	No
QA2.2	After visiting Toilet	Yes	No
QA2.3	Handling thobwa bottles	Yes	No
QA2.4	Packing the thobwa in bottles	Yes	No
QA2.5	Other	Yes	No

Other specify……

Section B: Environmental hygiene (*ukhondo pamalo ozungulira***): *observe the market surrounding and check if they have the following points. Check also if they use them by asking the respondent (thobwa sellers).* *(Yang'anani malo ozungulira pamsika ndikuyankha mafunso otsatirawa).***

QB1: Condition of the market (hygienically good-this is a market area which has been either swept clean *(ukhondo wa pamsika)*

QB1.1	Clean and Well-kept place	Yes	No
QB1.2	Sheltered from dust, sun, rain and wind	Yes	No
QB1.3	Far from all sources of contamination, solid waste	Yes	No
QB1.4	Sweep the ground	Yes	No
QB1.5	Clean Environment (Garbage's, Dirty, Visible dust)	Yes	No
QB1.6	Clean spills when they occur	Yes	No
QB1.7	Clean garbage disposal, Dust bin tightly closed	Yes	No

Any other comments ……..

QB2: Is there a handwashing facility available at Market place? How is the condition of the handwashing facility? *(Pali chosambira mmanja malo ogulisirapo chakudya?*

QB2.1	Availability of handwashing facility	Yes	No	Not Applicable
QB2.2	Functionality of handwashing facility	Yes	No	Not Applicable
QB2.3	Water inside the hand washing facility	Yes	No	Not Applicable
QB2.4	Can handwashing facility effectively be used	Yes	No	Not Applicable

QB3: What about workbenches/counters, raw ingredient storage, evidence of rodents and insects, doors and windows open to the environment?

QB3.1	Keep everything safely and securely	Yes	No
QB3.2	Bottles and Cups should be carefully stored and carried	Yes	No
QB3.3	Shade and covers area	Yes	No
QB3.4	Wipe clean surfaces on all food selling point area	Yes	No
QB3.5	Keep surfaces utensils clean	Yes	No
QB3.6	Use double bowls/ sink for washing and rinsing	Yes	No
QB3.7	Using hot water or washing up liquid, for washing all equipment including bottles	Yes	No
QB3.8	Ensure that adequate bucket are cleaned or replaced regularly	Yes	No

QB4: Availability of animals (pets) like cats, dogs, chickens, etc. and their access to the market or selling point

QB4.1	Protect Thobwa from flying insects	Yes	No	Not Applicable
QB4.2	Protect Thobwa from Cats	Yes	No	Not Applicable

QB4.3	Protect Thobwa from Dogs	Yes	No	Not Applicable
QB4.4	Protect Thobwa from Chickens	Yes	No	Not Applicable
QB4.5	Protect Thobwa from Goats	Yes	No	Not Applicable

Other ……

QB5: Availability of drainage for wastewater and its hygienic condition *(Ngalande yotayira madzi-ikuoneka motani)*

QB5.1	Is drainage system available	Yes	No	
QB5.2	Does the drainage system covered, (Use running water system properly)	Yes	No	Not Applicable
QB5.3	Does the drainage system clean	Yes	No	Not Applicable
QB5.4	Adequate Bins and Bags (covered, bags tied when full) for all waste	Yes	No	Not Applicable

QB6 Type of Toilet/latrine usage at the place (please check on the type of latrine and availability of drop hole cover, whether the toilet is shared or not. Is there anal cleansing materials inside the toilet?) *(chimbudzi chogwiritsa ntchito)*

QB6.1	Availability of Toilet	Yes	No	
QB6.2	Availability of Roofing at the Toilet	Yes	No	
QB6.3	Flush toilet & septic tank	Yes	No	Not Applicable
QB6.4	Pour flush toilet & septic tank	Yes	No	Not Applicable
QB6.5	Pour Flush and Pit	Yes	No	Not Applicable
QB6.6	Pour flush with concrete slab	Yes	No	Not Applicable
QB6.7	Concrete slab & pit	Yes	No	Not Applicable
QB6.8	Wood board & pit	Yes	No	Not Applicable
QB6.9	Urine diverting toilet	Yes	No	Not Applicable
QB6.10	Locally smeared latrines	Yes	No	Not Applicable
QB6.11	Drop hole cover	Yes	No	Not Applicable
QB6.12	Ventilation pipe	Yes	No	Not Applicable
QB6.13	Faeces visible on the floor	Yes	No	Not Applicable
QB6.14	Faeces visible on the walls	Yes	No	Not Applicable

Other(Specify)……

QB7 If the toilet is not available, where do they defecate? (*ngati chimbudzi palibe, mumakadzithandizira kuti?)*

QB7.1	Bush *(mutchire)*	Yes	No
QB7.2	River *(mumtsinje)*	Yes	No
QB7.3	Other household latrine *(kwa aneba)*	Yes	No

Others (specify)*(zina:fotokozani* ……..

QB8: Is there a handwashing facility available, close to the toilet? How is the condition of the handwashing facility? *(Pali chosambira mmanja pafupi ndi chimbudzi?*

QB8.1	Availability handwashing facility	Yes	No	
QB8.2	Functionality handwashing facility	Yes	No	Not applicable
QB8.3	Water inside the hand washing facility	Yes	No	Not applicable
QB8.4	Can handwashing facility effectively be used	Yes	No	Not applicable
QB8.5	Availability of handwashing material (Soap/Ash)	Yes	No	Not applicable

QB9 Observe the general outlook and hygienic conditions of the containers used to keep thobwa at selling point. (*yang'anitsitsani ziwiya zomwe zikugwiritsidwa tchito pamsika pogulitsa thobwa.)*

Conditions of the containers used to keep Thobwa at Selling point

QB9.1	Plastic Pail with a cover	Yes	No
QB9.2	Cup Storage container with covers	Yes	No
QB9.3	Bottle Storage pail with covers	Yes	No
QB9.4	Other		

Other specify……

QB10: Observe the source of water during thobwa selling at the market

QB10.1	Standing water (Piped)	Yes	No
QB10.2	Surface water (lake, pond, basin, stream, river)	Yes	No
QB10.3	Ground water (Well, Borehole)	Yes	No
QB10.4	Other	Yes	No

Other specify……..

QB11: Observe how the thobwa get carried to the selling point. (*yang'anitsitsani momwe thobwa likunyamulidwa kupita nalo kumsika.)*

QB11.1	In a covered pail	Yes	No	Not Applicable
QB11.2	In a cooler box (already packaged)	Yes	No	Not Applicable
QB11.3	In a pail (already packaged)	Yes	No	Not Applicable

QB12: Observe the general parking of the final product and hygienic conditions of the bottles used to park thobwa at selling point. (*yang'anitsitsani ziwiya zomwe zikugwiritsidwa tchito posugira thobwa Pamsika)*

QB12.1	Bottles bought from the shops	Yes	No	Not applicable
QB12.2	From a local supplier (Recycled bottles)	Yes	No	Not applicable
QB12.3	Reused Bottles (Sourced by self/Children)	Yes	No	Not applicable
QB12.4	Cup bought from the shops	Yes	No	Not applicable
QB12.5	General cleanliness and disinfection of cups	Yes	No	Not applicable

General comments and other observations (*Ndemanga ndi zooneka zina ku Msika):*

……
……

End of observation checklist at selling point

Appendix 4: Information sheet (English & translated version)

INFORMATION SHEET

The study on assessment on the levels of contamination of "Thobwa" and how it can be minimized using the HACCP system

My name is …….., and I am here on behalf of Phillimon Phiri, a student of Master's Degree in Environmental health at Polytechnic under the University of Malawi. As part of partial fulfilment of the studies, Phillimon is conducting the research to assessment the levels of contamination of Thobwa and how it can be minimised using the HACCP system. Participation to this study is voluntary and this study is targeting men and women who are above the age of eighteen, who are thobwa sellers, located in various outlets within Blantyre city. I would therefore like to interact and learn from you on how Thobwa is prepared and parked before it reached the intended user at the market. Included in the interaction will be to appreciate some of the risks which might be associated with the preparation and handling process.

As participation is voluntary, no incentives are to be provided to the respondents and data collected shall sorely be used for study purposes. No names of respondents shall be collected in the course of interaction.

More information

If you have any question now or after the interview concerning the study, you may contact the Principle Investigator Mr Phillimon Phiri on +265 888 850 285 or Mr. Save Kumwenda on +265 888 389 452.

Chichewa Translated information sheet

INFORMATION SHEET

The study on assessment on the levels of contamination of "thobwa" and how it can be minimized using the HACCP system

Dzina langa ndine…… ndipo ndikuimilira mmalo mwa Phillimon Phiri wophunzira sukulu ku Polytechnic pansi ya sukulu ya Univesite ya Malawi. Monga mbali imodzi ya maphunziro, Phillimon akupanga kafukufuku wa ukhondo ndi chakudya makamaka thobwa. Kutenga nawo mbali pakafukufukuyi ndikwaufulu komanso mosakakmizidwa, kwa okhawo amene zaka zao zapyola khumi ndi zisanu ndi zitatu. (18), kwa iwo amene amaphika komanso kugulitsa thobwa mu nzinda wa blantyre. Potero, ndimafuna ndicheze nanu zokhudzana ndi nkhani ya thobwayi. Mkati mwakucheza, ndifunanso ndiphunzire mmene mumaphikira komanso kusamalira thobwa lisanapite pansika.

Kafukufukuyu ndiosakakamiza, ndipo kutenga nawo mbali ndimwakufuna kwanu komanso palibe phindu linalililonse lomwe lingadze pakutenga nawo mbali pa kafukufukuyu. Dziwani kuti palibe chiopsezo chinachilichonse chomwe chingadze pakutenga nawo mbali.

Kuonjedzerapo, dziwani kuti uthenga omwe mutapeleke pa nkhaniyi ukhala wachinsinsi ndipo palibe yemwe atanve nao za uthenga umenewu ndiponso dzina lanu sililembedwa paliponse. Ndinu omasuka kufunsa funsa lililonse kapena kusiya kutenga nawo mbali mu kafukufukuyu pa nthawi yomwe mwafunila

Uthenga Owonjezera

Ngati muli ndi funso kapena kufuna uthenga oonjezera pa kafukufukuyi mutha kuyankhulana ndi Mr Phillimon Phiri pa +265 888 850 285 kapena Mr. Save Kumwenda pa +265 888 389 452.

Appendix 5: Consent form (English & translated version)

CONSENT FORM

Concerning participation in the Research Project: Study on assessment of levels of contamination for thobwa among venders in Blantyre city, Malawi.

Principal Investigator & Institutional Affiliation : Phillimon Peter Phiri, MSc-EH Student The Polytechnic- University of Malawi

I understand that I have been invited to participate in an interview. I have heard the aims and objectives of the Research Project that are proposed. I have been given opportunity to ask questions and enough time to think about the Research Project. I have not been forced or pushed in any way to take part. I am clear about the aims of the Research Project.

I fully understand that participation in this Research Project is absolutely voluntary i.e. of my own choice. I am aware that I may withdraw from the participating in the study at any point without giving any reasons.

I also do understand that the interviews are being documented and will be used only for the purpose of analysing data in this Research Project. I have been informed that only the researchers will have access to the information being documented. I have also been told that when the analysis is complete the questionnaires will be destroyed.

I am aware that the results of this Research Project will be used for scientific and educational purposes and that may include being published. I agree to this, provided any identifying data of region and name if any are removed.

In case you would like to have further communication you can contact:

1. The Principal Investigator (P.P. Phiri)
   University of Malawi-The Polytechnic
   P/bag 303

   Chichiri

   BLANTYRE 3

   Cell: 0994 200 500/0888 850 0285

   Email: pphiri81@gmail.com

I hereby agree to voluntarily participate in interviews in this Research Project.

……..... .....

Name of participant Signature of participant

……..... …….....

Place Date .

Statement by the interviewer:

I have given written and oral information regarding this Research Project to the participant.

I agree to answer any future questions concerning the Project as best as I am able.

I will adhere to the protocol as it has been approved.

……..... ..... ……..... …...

Name of interviewer Signature Date Place

Chilolezo cha kafukufuku

Kutenga nawo mbali pa kafukufuku wa sukulu: kufuna kupeza mmene thobwa limaphikidwira komanso zovuta zomwe zingakhalepo ngati ukhondo utapanda kutsatidwa pamene anthu aphika kapena kugulitsa thobwa, mu nzinda wa Blantyre. Malawi.

Wotsogolera kafukufuku ndi sukulu yochokera: Phillimon Peter Phiri, MSc EH Student The Polytechnic- University of Malawi

Ndapemphedwa kutenga nawo mbali pa kafukufukuyi. Ndamvetsetsa zolinga za kafuku ameneyu, ndipo ndapatsidwa mpata wofunsa mafunso ndikuganizira zakafukufuku ameneyi. Sindinakakamizibwe mwanjira iliyonse kutenga nawo mbali mukafukufuku ameneyi.

Ndamvetsetsa kuti kutenga nawo mbali mkafukufuku ameneyi ndimodzipereka komanso ndikhonza kusiya kuyankha panthawi ina iliyonse pamene ndaona kuti sindikumvetsetsa zones zomwe tikambirane mukafukufuyi zidzagwiritsidwa nthiot pa nkhani yakafukufuka basi, ndipo plebe ndina la wina aliyense limene lidzatchulidwa mkati mwa kafukufuku ameneyi.

Ngati pali zofunsa zina, lankhulani ndi:

1. The Principal Investigator (P.P. Phiri)
   University of Malawi-The Polytechnic
   P/bag 303

   Chichiri

   BLANTYRE 3

   Cell: 0994 200 500/0888 850 0285

   Email: pphiri81@gmail.com

Ndavomera kutenga nawo mbali pakafukufuku.

……..... .....

Dzina Sayini/chidindo cha chala

……..... …….....

Malo Tsiku .

Mau ochokera kwa ofunsa mafunso:

Onse amene akutenga mbali mukafukufukuyi, achita mwakufuna kwao, atapemphedwa kutero, komanso atapatsidwa ndondomeko zones zakafukufuku.

Ngati wina alindi mafunso kapena sakuvetetsa, ndidzayesetsa kufokotokoza momveka bwino.

….....	.....	…….....	…….....
Dzina	Siyini	Tsiku	Malo

Appendix 6: Letter of approval from national research and ethical committee

Telephone: + 265 789 400
Facsimile: + 265 789 431

All Communications should be addressed to:

The Secretary for Health and Population

In reply please quote No.

.....

MINISTRY OF HEALTH AND POPULATIO

P.O. BOX 30377
LILONGWE 3
MALAWI

9th May, 2018

Phillimon Peter Phiri
Malawi Polytechnic Washted

Dear Sir/Madam,

Re: Protocol # 18/04/2018: An Assessment on the Levels of Contamination of Thobwa and How It Can Be Minimized Using the HACCP System in Blantyre City Venders

Thank you for the above titled proposal that you submitted to the National Health Sciences Research Committee (NHSRC) for review. Please be advised that the NHSRC has **reviewed** and **approved** your application to **conduct** the above titled study.

- **APPROVAL NUMBER : 2018**
- The above details should be used on all correspondences, consent forms and documents as appropriate.
- **APPROVAL DATE : 09/05/2018**
- **EXPIRATION DATE**
  This approval expires on **08/05/2019**. After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the NHSRC Secretariat should be submitted one month before the expiration date for continuing review.
- **SERIOUS ADVERSE EVENT REPORTING:** All serious problems having to do with subject safety must be reported to the NHSRC within 10 working days using standard forms obtainable from the NHSRC Secretariat.
- **MODIFICATIONS**: Prior NHSRC approval using forms obtainable from the NHSRC Secretariat is required before implementing any changes in the protocol (including changes in the consent documents). You may not use any other consent documents besides those approved by the NHSRC.
- **TERMINATION OF STUDY:** On termination of a study, a report has to be submitted to the NHSRC using standard forms obtainable from the NHSRC Secretariat.
- **QUESTIONS**: Please contact the NHSRC on phone number *+265 888 344 443* or by email on mohdoccentre@gmail.com.
- **OTHER**: Please be reminded to send in copies of your final research results for our records (Health Research Database).

Kind regards from the NHSRC Secretariat

SECRETARY FOR HEALTH
2018 -05- 09

For: **CHAIRPERSON, NATIONAL HEALTH SCIENCES RESEARCH COMMITTEE**

Promoting Ethical Conduct of Research[1]

Executive Committee: *Dr B. Chilima (Chairperson), Dr B. Ngwira (Vice-Chairperson)*
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Appendix 7: Laboratory protocols for sample collection and analysis.

Coliform test: Most Probable Number (MPN) method

The most probable number (MPN) technique is an important technique in estimating microbial populations in soil, water, and agricultural products. Many soils are heterogeneous; therefore, exact cell numbers of an individual organism can be impossible to determine. The MPN technique is used to estimate microbial population sizes in situations like this. The technique does not rely on quantitative assessment of individual cells, instead it relies on specific qualitative attributes of the microorganism being counted. The important aspect of MPN methodology is the ability to estimate a microbial population size based on a process-related attribute. This is also another protocol to be considered for some of the samples collected for results validation.

The MPN technique estimates microbial population sizes in a liquid substrate. The methodology for the MPN technique is dilution and incubation of replicated cultures across several serial dilution steps. This technique relies on the pattern of positive and negative test results following inoculation of a suitable test medium (usually with a pH sensitive indicator dye). Here is the general equation for determining the MPN of organisms in a substrate after it is serially diluted and several units are inoculated of each dilution

Materials required

- MacConkey Broth
- 20 ml McCartney bottles
- 10ml test tubes
- 5 ml test tubes
- Durham tubes
- 250ml sampling bottles
- 20ml pipettes
- 10ml pipettes
- 5ml pipettes
- 1ml pipettes
- 0.1ml pipettes
- Balance
- Incubator set at 37^{o}C

Procedure

a. 50ml of water sample to 50ml of double- strength MacConkey broth.

b. 10ml of water sample to each of 5 tubes of 10ml of double-strength MacConkey broth.
c. 1ml of water sample to each of 5 tubes of 5ml of single-strength MacConkey broth.
d. 0.1ml of water sample to each of 5 tubes of 5ml of single-strength MacConkey broth

Note: include a Durham tube in each McCartney bottle.

Incubate at 35-37^{o}C and note the numbers of tubes showing acid and gas at 48 hours. Tap tubes showing no gas. A bubble may then form in the Durham tube. Consult the MPN tables (tables 11.1-11.3 **pages 205-210** in Microbiological Methods fourth edition) and read the most probable number of presumptive Coliform bacilli per 100ml of water sample. Small amounts of gas occurring after 48 hours in presumptive tubes are disregarded unless the presence of Coliform bacilli is confirmed by plating.

Part 2. E.coli confirmation test-Indole test

From each tube showing acid and gas, inoculate a tube of Tryptone water broth and incubate at 44^{o}C in a reliable water bath for 24 hours. Only Escherichia coli produces gas and Indole at 44^{o}C

3M Petrifilm E. coli /Coliform Count Plate

To be used for the enumeration of *Escherichia coli* and coliforms.

The 3M Petrifilm *E. coli* Count Plate is a reliable, sample-ready medium system for enumerating *Escherichia coli* and other coliforms. Petrifilm *E. coli* Count Plates contain violet red bile nutrients, a cold water soluble gelling agent, a glucuronidase indicator to identify *E. coli,* and a tetrazolium indicator to enhance the visualization of other gram coliform negative (non-*E. coli*) bacteria.

Coliforms ferment the lactose in the medium to produce gas. This gas is trapped around the coliform colony and allows the differentiation of coliform bacteria from other gram negative bacteria. In addition, glucuronidate, produced by most *E. coli* will react with the glucuronidase indicator in the medium to produce a blue precipitate around the colony allowing visual identification of *E.coli*.

Storage and disposal:

Store unopened Petrifilm plate foil pouches at or below 80C (460F). After opening, return unused plates to foil pouches. Seal foil pouch by folding and taping the open end. Store resealed pouches at room temperature (<25C or <77F) with <50% RH. Exposure of Petrifilm plates to higher temperatures and/or humidity can affect the performance of the plates. Do not refrigerate opened packages. Use Petrifilm plates within one month after opening. Do not use plates that show orange or brown discoloration. After use, Petrifilm *E.coli* Count Plates will contain viable bacteria.

Directions for use:

1. Place the Petrifilm *E.coli* Count Plate on a flat surface.
2. Lift top film and dispense 1 ml of sample onto the centre of the bottom film.
3. Slowly roll the top film down onto the sample to prevent the entrapment of air bubbles.
4. Distribute sample evenly within the circular well using a gentle downward pressure on the centre of the plastic spreader. Do not slide spreader across the film. Remove spreader and leave plate undisturbed for one minute to permit solidification of the gel.
5. Incubate plates in a horizontal position with the clear side up in stacks not exceeding 20 plates. Incubate plates 24 $\pm$ 2 hr. and examine for coliform and *E. coli* growth. Some *E. coli* colonies require additional time to form the blue precipitate. Re-incubate plates an additional 24 $\pm$ 2 hr. to detect any additional E. coil growth.
6. Petrifilm *E. coil* Count Plates can be counted on a standard colony counter. The Interpretation of *E. coli* colonies on Petrifilm *E. coli* Count Plates varies by method. When considering which interpretation to follow it will be noted that approximately 95% of *E. coil* produce gas.

AOAC Method (991.14)

Petrifilm plates incubated at 35 $\pm$ 1C. Blue colonies associated with entrapped gas are confirmed *E. coli*. Blue colonies without gas are not counted as *E. coil.* Other coliform colonies will be red and associated with gas bubbles. Colonies not associated with gas (with a distance greater than one colony diameter from gas bubble) are not counted as coliforms. The total coliform count consists of both the red and blue colonies associated with gas at 24 hours.

AFNOR Method (01/4 - 09/92)

Petrifilm plates incubated at 44.5 $\pm$ 0.5C. Blue colonies with gas are *E. coil*. Blue colonies without gas may or may not be *E. coil* and may be confirmed if necessary. An important aspect

to take note is that that *E. coli* 0157:H7 does not grow at ≥ 44.5C and is glucuronidase negative and therefore will not produce a blue precipitate. This is going to be confirmed on the 3M Petrifilm test kit-HEC to identify which one is *E. coil* 0157:H7. Do not count colonies on the foam dam since they are removed from the selective influence of the medium. The circular growth area is 20 cm2. Estimates can be made on plates containing greater than 150 colonies by counting a representative number of squares and multiplying by the appropriate number to obtain an estimated count for the total 20 cm2 growth area.

b. High concentrations of *E. coli* will cause the growth area to mm a bluish colour while high concentrations of coliforms (non-*E. coli*) will cause the growth area to turn a dark reddish colour. When this occurs, further dilution of the sample is required to obtain a more accurate count. To isolate colonies for further identification, lift the top film and pick the colony from the gel.

Standard Operating Procedure for detecting E.coli contamination in drinking water using IDEXX'S Colilert

Facility: Microbiology Laboratory, Pathology Department, College of Medicine, University of Malawi.

Laboratory Lead: Chisomo Msefula, Reenesh Prakash (Microbiology Building, College of Medicine, Mobile:) and Dr. Steve Taulo (Polytechnic, Mobile:)

Value of test: To detect and quantify coliforms in water sampled from a community water source and from household storage.

Last Revision:(date)

Principle of test:

Colilert detects both total coliforms and E. coli in water with a limit of detection at 1cfu/100mL. The bacteria metabolises Colilert's nutrient indicator, which fluoresces yellow. *E.coli* metabolises an additional nutrient indicator, which also fluoresces.

Sample collection:

1. Label a sterile 120mL glass bottle with sample Id, date and time of collection
2. Record in the sample collection book the type of water source
3. Record whether the water was previously boiled, chlorinated, filtered or any treatment done.

4. Record any identifiable sources of pollution in the area where water is sampled
5. Record temperature of the water source
6. Record if there are any external fittings if collecting water from a tap
7. Put on sterile gloves when opening the sterile bottle and collect the water

Transporting water samples to the Laboratory:

a. Place the water-filled 120mL glass bottle in an insulated cold box
b. Transport the samples within 6 hours of collection

Reagents and Equipment

- 120mL disposable vessels with sodium thiosulphate
- Colilert Quanti-Tray comparator
- Colilert Quanti-Tray
- IDEXX sealer
- 6-watt, 365-nm UV light
- Sterile, transparent, non-fluorescing 100mL Colilert bottles
- 200mL glass bottles
- Autoclave
- 70% ethanol
- Virkon
- Gloves

Bacteriological examination of water:

1. Decontaminate the working bench with Virkon and 70% ethanol
2. Aseptically add 100mL of water sample to a colilert bottle
3. Add contents of one pack to a 100 mL water sample in a sterile vessel.
4. Cap vessel and shake until dissolved.
5. Pour sample/reagent mixture into a Quanti-Tray or Quanti-Tray/2000 and seal in an IDEXX Quanti-Tray Sealer
6. Place the sealed tray in a 35±0.5°C incubator for 24 hours.
7. Read results according to the Result Interpretation table below.
8. Count the number of positive wells and refer to the MPN table provided with the trays to obtain a Most Probable Number.
9. Look for fluorescence with a 6-watt, 365-nm UV light within 5 inches of the sample in a dark environment. Face light away from your eyes and towards the sample.
10. If the results are ambiguous based on the initial reading, incubate up to an additional four hours (but not to exceed 28 hours total) to allow the color and/or fluorescence to intensify.
11. Positives for both total coliforms and *E. coli* observed before 24 hours and negatives observed after 28 hours are also valid.

*Taken from IDEXX'S Colilert Test procedure document, including the table below.

Appearance	**Result**
Less yellow than the comparator1	Negative for total coliforms and E. coli
Yellow equal to or greater than the comparator	Positive for total coliforms
Yellow and fluorescence equal to or greater than the comparator	Positive for E. coli

Standard Operating Procedure for detecting coliforms, E.coli, Salmonella, and Staphylococcus cloth, utensils, food prep surface, container contamination using 3M™ Petrifilm™

Principle of test:

Facility: Microbiology Laboratory, Pathology Department, College of Medicine, University of Malawi.

Laboratory Leads: Chisomo Msefula, Reenesh Prakash (Microbiology Building, College of Medicine, Mobile:) and Steve Taulo (Polytechnic, Mobile:)

Value of test: To detect and quantify *E.coli, Salmonella* and *Staphylococcus* from swabs and rinse water of cloth that is used for carrying/wrapping child

Last Revision: (date)

3M™ Petrifilm™ allows the identification and count of bacterial colonies using an indicator dye and a built in grid. Further tests of the colonies to confirm the bacteria genus and species are done.

Sample collection:

A. Using a sterile cotton swab pre-moistened in Buffered Peptone Water swab the surface up to approximately 5 x 5cm square
B. Transfer cotton swab into falcon tubes with peptone water.

Transporting samples to the Laboratory:

A. Place the sample in an insulated cold box

B. Transport the samples within 6 hours of collection.

Reagents and Equipment

1. Zip-lock bags
2. E.coli 3M™ Petrifilm™
3. Staphylococcus aureus 3M™ Petrifilm™
4. Salmonella 3M™ Petrifilm™
5. 70% ethanol
6. Virkon
7. Gloves
8. Buffered peptone water
9. Kovacs reagent
10. Tryptone water
11. Water bath
12. Selenite broth
13. Rappaport vassiliadis broth
14. XLD plates
15. Salmonella antisera (Poly-O and Poly-H)
16. API Biochemical Identification kit
17. Refrigerator
18. 1mL pipette tips
19. Pipette (1000uL)

Bacteriological examination of food samples:

1. Decontaminate the working bench with Virkon and 70% ethanol
2. Prepare 10-fold dilutions of the samples
3. Add 1mL of appropriate dilution to petri-film specific for each of the three microorganisms
4. Incubate the petri-film at 37^{o}C for 24 hours
5. Culture presumptive E.coli colonies in tryptone water at 44^{o}C for 18 – 24 hours and perform an indole test with Kovac's reagent
6. Confirm Staphylococcus colonies by observing yellow colouration on mannitol salt agar after incubation for 24 hours at 37^{o}C
7. Confirm Salmonella growth by growing colonies on XLD agar and resulting positive colonies subculture onto nutrient agar. Perform API biochemical test and serology with Poly-O and Poly-H antisera.
8. Transfer Salmonella colonies onto beads and store at -80^{o}C.

References

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