CHARACTERIZATION OF BACTERIAL AND FUNGAL CONTAMINANTS OF BLACK CUT, TEAR & CURL AND GREEN TEAS (*CAMELLIA SINENSIS*) FROM SELECTED FACTORIES IN KENYA.

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A Thesis Submitted to the Board of Graduate Studies in Partial Fulfilment of the Requirements for the Award of the Degree of Master of Science in Microbiology of the University of Kabianga

UNIVERSITY OF KABIANGA

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DECLARATION AND APPROVAL

DECLARATION

This thesis is my original work and has not been submitted for the award of a diploma or conferment of a degree in this or any other institution.

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APPROVAL

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DEDICATION

This thesis is dedicated in a special way to my parents Mr. Philip Lang'at and Mrs. Nancy Lang'at and my siblings:-Robert, Collins, Linda and Gilbert.

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ABSTRACT

Tea (*Camellia sinensis*) is considered a low risk food in terms of microbial contamination because of the way it is processed, packaged and consumed. It is the most popular and widely consumed beverage worldwide, only after water. Its popularity is mainly due to its refreshing taste, attractive aroma, and potential health benefits such as antioxidant activity, ability to improve oral health, antibacterial, antifungal, and antiviral properties. However, there are possibilities of microbial contamination along the value chain and for this reason care should be taken to eliminate them. The primary objective was to develop comprehensive microbial quality control strategies aimed at minimizing cross-contamination risks during and after tea processing. By identifying and quantifying microbial populations at each processing stage, this study aimed to contribute valuable insights that could inform the implementation of effective hygiene protocols and ensure the production of microbiologically safe teas for consumers. This study focused on both cut, tear, and curl (CTC) and green orthodox tea processing steps. Key objectives included profiling microbes along the steps, evaluating microbial quality in black CTC teas per KS EAS 65:2018, identifying heat-resistant microbes post-brewing and assessing aflatoxin levels. Microbial assays encompassed total plate counts for yeast and moulds, *Escherichia coli, Salmonella spp.* and *Staphylococcus aureus*, detection of heat-resistant microbes and aflatoxin-producing fungi. Made tea samples consisting of primary and secondary tea grades from the drier mouth and bins of 14 pre-selected Kenya Tea Development Agency (KTDA) tea factories from the East and West of Rift Valley tea growing areas were collected. In-process teas from a private factory and a multinational company were also assayed up to the finished product stage. The microbial quality status of teas were assayed as stipulated in Kenyan Black Tea Standard and ISO protocols and checked for conformance. Heat-resistant microorganisms in tea which survived brewing in hot or boiling water of 90-100 °C were assayed. Bacteria and fungi were isolated using Nutrient Agar (NA) and Potato Dextrose Agar (PDA). Aflatoxin levels were quantified using High Performance Liquid Chromatography (HPLC) following Thin Layer Chromatography (TLC) screening. The key findings of this research study included the isolation of *Escherichia coli* and *Staphylococcus spp.* from tea samples, while *Salmonella spp*. was absent in the made tea samples. This research study also revealed the existence of heat-resistant bacteria in the black CTC tea and this causes great concern as they may pose a health risk. Among them were some heat-resistant *E. coli* and heat-resistant *staphylococcus aureus*. Stored black teas showed acceptable levels of yeast and moulds, although contamination was observed. This research study addressed the gap in existing research regarding the microbial status of teas across various stages of processing in Kenyan tea factories, encompassing leaf reception, withering, maceration, oxidation, drying, sorting and grading, packaging and storage. Prior to this investigation, no documented research had systematically examined microbial contamination throughout these critical processing stages. Some teas did not meet microbial quality standards, highlighting the need for improved handling practices in certain factories. Importantly, teas from selected KTDA factories were generally free from aflatoxins, underscoring satisfactory control measures in place for mycotoxin management. This study also identified critical control points in tea processing to enhance microbial safety and recommended operational procedures for processing, sorting, packaging, storage and brewing. The findings confirm the microbial safety of processed tea for human consumption, providing insights into the diversity and quantity of microbes identified.

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LIST OF ABBREVIATIONS AND ACRONYMS

AFA-TD	Agriculture and Food Authority -Tea Directorate
AIDS	Acquired Immune Deficiency Syndrome
ANOVA	Analysis of Variance
BEA	Bile Esculin Agar
BT	Black Tea
BP	Broken Pekoe
CAC	Codex Alimentarius Commission
CFU	Colony Forming Units
CNS	Central Nervous System
CTC	Cut, Tear and Curl
CVD	Cardio Vascular Disease
D	Dust
DM	Drier Mouth
DNA	DeoxyriboNucleic Acid
EAS	East African Standards
ECG	Epicatechin gallate
E. coli	*Escherichia coli*
EGC	Epigallocatechin
EGCG	Epigallocatechin Gallate

EHEC	Enterohemorrhagic *E. coli*
F	Fannings
FBD	Fluid Bed Drier
GABA	Gamma Amino Butyric Acid
GAPs	Good Agricultural Practices
GDP	Gross Domestic Product
GMPs	Good Manufacturing Practices
Gp 41	Glycoprotein 41
GT	Green Tea
HACCP	Hazard Analysis Critical Control Point
HC	Hemorrhagic Colitis
HEK 293 WT cells	Human Embryonic Kidney 293 Wild Type cells
HPLC	High Performance Liquid Chromatography
ISO	International Organization for Standardization
KEBS	Kenya Bureau of Standards
KS	Kenya Standard
KTDA	Kenya Tea Development Agency
LDH	Lactate Dehydrogenase
LF	Lactoferrin
LSD	Least Significant Difference Test

MPN	Most Probable Number
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSA	Mannitol Salt Agar
NACOSTI	National Commission for Science, Technology and Innovation
OSHA	Occupational Safety and Health Act
PD	Pekoe Dust
PF	Pekoe Fannings
POD	Polyphenol Peroxidase
PPB	Pharmacy and Poisons Board
PPE	Personal Protective Equipment
PPO	Polyphenol Oxidase Enzyme
QC	Quality Control
RAPD	Random Amplified Polymorphic DNA
RF	Retention Factor
SAS	Statistical Analysis Software
S. aureus	*Staphylococcus aureus*
SIM	Sulfur Indole Motility Media
SOPs	Standard Operating Procedures
SPC	Standard Plate Count
TMF	Tailored Mixed Fannings

TFs	Theaflavins
TLC	Thin Layer Chromatography
TRI	Tea Research Institute
TRFK	Tea Research Foundation of Kenya
TRs	Thearubigins
XLD	Xylose Lysine Deoxycholate

CHAPTER ONE

INTRODUCTION

1.1 Overview

This thesis reports findings of a research study undertaken to determine the microbial quality of made teas from selected Kenyan tea factories. Made tea is a term used in the tea industry referring to processed tea which is an outcome of transforming green leaf. The background of the study is well captured in this chapter and it breaks down the foundations that form the basis of this study. The statement of the problem is also well outlined in this section and a clear emphasis is placed on the significance of the study. The objectives of the study and the expected output of the research study that was undertaken have also been outlined in this chapter.

1.2 Background Information

Tea (*Camellia sinensis, Theaceae*) is the most popular beverage in the world, consumed more widely than any other drink, except for water. This wide consumption worldwide owed to its attractive aroma, refreshing taste and the potential health benefits it has (Hodgson and Croft, 2010). These potential health benefits include: - antioxidant activity, ability to improve oral health, antibacterial, antifungal and antiviral activities (Chen et al., 2019). Tea was first utilized as herbal medicine in the Yunnan province of South Western China. Tea consumers are currently more health conscious thus demanding pharmacologically active tea products. Habitual consumption helps combat a variety of diseases including: - several cancer types, diabetes, vomiting, clastogenesis, Inflammation

and cardiac problems (Arnadi et al., 2015). However, in order to get these health benefits, consumers need to start by taking safe teas.

Kenya produces 22% of the world's black tea, placing it third next to China and India in terms of tea production (ITC, 2009). This Kenyan tea is usually exported to countries such as Pakistan, UK, Egypt, Afghanistan and Sudan among others (Yego et al. 2016). The export makes up a total of 95%, whereas the percentage that is consumed locally is 5%. Tea as an agribusiness contributes up to 4% of the Kenya's GDP, making it the largest sector and one of the top foreign exchange earners after tourism and horticulture, at 26% of the total earnings of foreign. The tea industry remains one of the few profitable agro-enterprises in Kenya and it has expanded greatly over the 80 years of its existence and the country is now the largest single exporter of tea (Wachira and Ronno, 2005; Owuor and Obanda, 2007). As a whole, the tea industry is also a source of livelihood for 3 million Kenyans directly and indirectly along its value chain.

Tea is grown in regions with mountainous terrain where favourable climate and rich soil is found. Upon harvesting from the tea garden and prior to being processed, tea leaves and buds are not washed or cleaned (Hossain et al., 2013). Storage conditions that are dry, cold, dark, and inert are critical in the preservation of made tea. The handling, packaging and storage after drying may result in microbial contamination of the processed tea leaves even though the final drying stage at about 120 °C is adequate to reduce the high bacterial and fungal load (Bouakline et al., 2000; Wilson et al., 2004). Tea is best made by pouring hot water over dried *Camellia sinensis* leaves. Nevertheless, using water at sub-boiling temperatures may not eradicate all contaminants, including bacterial spores (Sonenshein et

al., 2000). Green and white teas are preferably infused with sub-boiling water to preserve its flavour (Astill et al., 2001). Exposure to microorganisms, which maybe pathogenic may occur during production and storage, thus during consumption, the infusion may be hazardous to one's health (Wilson et al., 2004). This will also result in the reduction in quality and ultimately the demand for tea brands (Dayananda et al., 2017).

Characterization of microbes involves identifying their physical and biochemical properties, such as shape, size and metabolic processes. Techniques including microscopy, culture methods and molecular biology tools such as DNA sequencing,are employed to understand their taxonomy and ecological roles.Aflatoxins and fungal colonies have been detected in teas due to contamination from plantation and working conditions in factories (Michiharu et al., 2008). Most microorganisms in nature are beneficial to human health, with only a few being harmful, causing spoilage, and infection. Fungi such as *Penicillium* are very widespread and common during post-fermented tea storage, are vital in inhibiting growth of spoilage organisms and even infectious bacteria during storage of post-fermented teas and also give distinct aroma and quality to the teas (Fang et al., 2008, Haas et al., 2013). Despite manufactured tea being a potential host of microorganisms, millions around the world drink at least one cup of tea daily (Hossain et al., 2013).

The Kenya’s tea exports to top buyer Pakistan in 2018, dropped by half, this was an equivalent of US$36.55 million loss in sales compared to the same time frame the year before. This was as a result of the top market instituting a requirement for rigorous aflatoxin tests in the Daily Nation newspaper reports in the year 2018. This decline in sales was attributed to strict rules used in aflatoxin tests. The Tea Directorate of Agriculture and Food

Authority (AFA-TD) attributed to strict rules used in aflatoxin tests following a disagreement between Kenya and Pakistan over suspected aflatoxin in local tea as reported by The Daily Nation in 2018. Due to uncertainties, the buyers became reluctant to purchase Kenyan made tea. Plant Protection Board of Pakistan had directed that all tea imports from Kenya be subjected to compulsory aflatoxin tests.

Thus, the study purposed to assess the microbial quality of Kenyan made teas (both black and green teas). Black tea undergoes fermentation (aeration) whereas green tea does not (e et al., 2015). The study will also seek to find out if there is a difference in their microbial contents. Manufacturing of green tea is quicker since fresh leaves from plantations undergo pan-frying or steaming so as to deactivate polyphenol oxidase and peroxidase (oxidizing) enzymes leading to polyphenol retention (Ahmed and Stepp, 2013). The tea then undergoes maceration and is finally dried. Owing to less processing steps, green tea will be expected to be the least contaminated during the stages of processing. Black tea processing takes longer because it involves withering and aeration to facilitate enzymatic oxidation of polyphenolic compounds and subsequent condensation resulting in formation of theaflavin (TF) as well as thearubigin (TR) (Zhang et al., 2013; Soni et al., 2015).

1.3 Statement of the Problem

Tea processing and consumption methods ensures least microbial contamination and reports indicate that some Kenyan teas fail microbial quality tests. In Kenya, little research work and documentation has been made on the microbial quality and safety of processed teas compared to numerous work done on microbial plant and soil health thus necessitating this study. There is a risk of contamination of made teas during handling from the leaf

harvesting through processing to consumption .The tea leaves are also potentially contaminated by environmental dust that settle on the different parts of the tea plant and could be containing bacterial and mould spores. *Staphylococcus aureus* can be transferred from humans to teas during the plucking, handling and packaging operation. This necessitates interrogating the tea processing steps from harvesting to processing within the factory and packaging in order to determine the critical points upon which contamination may occur. This will in turn ensure that quality of Kenyan teas is maintained and will thus re-establish consumer confidence. There is a general lack of knowledge about the microbiological status of made teas. Some Kenyan teas destined for the export market have been allegedly rejected due to the suspicion of the presence of pathogenic microorganisms. In some instances, consumers prepare iced teas by dipping tea bags in hot water or cold water as opposed to using extracts which is a potential risk for microbial contamination. The fear of contracting throat cancer from hot tea has caused some people to shift to drinking less hot tea or to using sub-boiling water for infusion thus causing potential risk of pathogenic microbial contamination. There's emergence of harmful heat-resistant pathogenic microbes in other foods thus the study sought to find out if these were present in tea by investigating the surviving microbes in processed teas after brewing.

There is no Kenyan standard for aflatoxiginic food safety hazards related to tea but the Uganda National Bureau of Standards, UNBS (2013) suggested that the maximum content of total aflatoxin in herbal tea products should not exceed 10 µg/kg in accordance to their herbal tea – specification. Black and speciality teas could be microbiologically contaminated during processing and packaging thus facilitating their contamination with

mycotoxins. The aflatoxin contamination is promoted by the aspect of black tea being a good substrate for the production of aflatoxins by the fungus *Aspergillus flavus*.

1.4 Objectives

1.4.1 General Objective

To characterize bacterial and fungal contaminants of black cut, tear and curl and green teas (*Camellia sinensis*) from selected factories in Kenya.

1.4.2 Specific Objectives

i. To isolate and enumerate bacterial load in raw material, in-process and finished products of black CTC and green teas from selected tea factories in Kenya.
ii. To isolate and characterize pathogenic bacteria in processed made black CTC teas from selected KTDA tea factories and determine their presence after brewing as per manufacturer's instructions.
iii. To isolate and characterize yeast and moulds in-processed made black CTC teas from selected KTDA tea factories.
iv. To determine the presence aflatoxin in made black CTC teas contaminated with yeast or moulds from selected KTDA tea factories.

1.5 Hypotheses of the Study

i. Raw material, in-process and finished black CTC and green teas from selected tea factories in Kenya have different bacterial load.

ii. There are no pathogenic bacteria in processed made black CTC teas from selected KTDA tea factories before and after brewing.

iii. There are no yeast and moulds in processed made black CTC teas from selected KTDA tea factories

iv. Aflatoxins are not present in made black CTC teas contaminated with yeast or moulds from selected KTDA tea factories.

1.6 Justification of the Study

This study was aimed at determining the adequacy of processing steps in black CTC and green tea processing to eliminate unwanted microbes to ultimately deliver safe tea products. Research on this domain would help to validate the safety of Kenyan teas and improve on measures to control and eliminate contamination. Understanding microbial risks can help in establishing effective monitoring and control measures, reducing the likelihood of contamination. The study also determined the effectiveness of brewing instructions prescribed by manufacturers in eliminating microorganisms in tea at the point of consumption. This is in accordance with a previous study which found out that during tea liquor preparation, boiling significantly lowered the levels of microbial load to levels that were safe for human consumption (Hossain et al., 2013).

This investigation sought to find out the levels of yeast and mould contamination across different KTDA factories in the East and West of the Rift Valley. This highlighted the critical role of handling and storage practices in microbial control. This would enhance consumer confidence in Kenyan exports. It is anticipated that this form of marketing would

make the Kenyan tea industry more sustainable and contribute to enhanced returns to tea producers and all stakeholders in the tea value chain including farmers, consumers, transporters, warehouse operators, investors and employees. This proactive approach can safeguard the industry's integrity and prevent economic losses related to product recalls. Focusing on the microbial quality of Kenyan tea not only safeguards health and enhances product quality but also strengthens market position and promotes sustainable agricultural practices in the tea sector.. A sustainable and highly profitable tea sector would contribute to Kenya's vision 2030(Gatimbu et al., 2020).

1.7 Expected Outputs

i. Documented information on how to enhance microbial safety of tea during processing and at storage in the Kenyan tea industry and information to guide consumers on the safety of Kenyan made teas for consumption.
ii. Dissemination of the findings to the tea industry stakeholders to aid in formulating policies and guidelines on safety of teas in Kenya.
iii. Publish research articles on tea microbial safety in peer reviewed journals.
iv. Generate data on microbial quality of Kenyan teas to help enhance compliance of Kenyan teas to international standards.
v. Thesis for MSc. degree award.

1.8 Significance of the Study

These health benefits of tea can only be experienced by consumers by first taking safe teas. This research profiled microorganisms, which are pathogenic that could occur during production and storage. These microorganisms could

potentially be hazardous to one's health during the infusion consumption of contaminated made teas. The findings of this research offer vital information that act as a base for confirming and documenting the safety of Kenyan made teas. The research findings would also ultimately serve in advising the tea stakeholders and the tea industry as a whole on measures to be undertaken and improvements needed to ensure that Kenyan made teas remain safe for human consumption. This would serve as a marketing point for Kenyan made teas both locally and internationally, seeing as 95% of Kenyan made teas are exported.

1.9 Scope of the Study

The current study investigated the microbial contamination of black and green made CTC teas from selected Kenyan tea factories. In determining the tea quality along the value chain a multinational and a private factory were selected. Tea samples were collected along the whole value chain from raw materials, in process to finished product and were collected aseptically from a multinational and a private factory and used for analysis of microbial quality.

In the determination of quality for processed teas, samples consisted of all the tea grades from the drier mouth and bins in a total of 14 pre-selected factories from the East and West of Rift Valley tea growing regions of the KTDA managed factories. This included factories from nine (9) tea growing counties - Kericho, Kiambu, Trans Nzoia, Kirinyaga, Narok, Nyamira, Meru, Nandi and Elgeyo Marakwet. The best and least performing factories were purposely selected from the Kenya Tea Development Agencies zonal demarcations of Kenya’s tea growing regions in the East and West regions of the Rift valley.

Those factories had the highest and lowest prices at the Mombasa auction in the year 2018. Microbial quality analysis of the teas was done at the TRI labs following storage the Tea Research Foundation Miniature Factory. The research work was done in seven months.

1.10 Limitations of the Study

The study had the following limitations:

a) Only the best and least performing Kenyan tea factories were selected leaving out moderate performers.

b) The scope of the microbes in the study was limited to only three types of bacteria, yeast and moulds.

c) Variability in environmental conditions, agricultural practices, and processing techniques.

d) Potential biases in sample collection, processing, and analysis methods.

1.11 Assumptions of the Study

This study assumed the following:

a) The microbial populations and processing conditions were a true representative of the actual conditions from selected tea processing factories.

b) Corrective actions are usually taken to eliminate non-conformance within the tea factories.

c) A routine microbial load assay analysis was being conducted in the selected tea factories with broadened objectives.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter presents information on literature reviewed in line with the title and objectives of the study. It set the study subject in a broader perspective through investigation of relevant literature and work done in relation to the study.

2.2 Review of Related Literature

2.2.1 History of Tea

Tea is becoming increasingly popular around the world due to its appealing flavour and health benefits to humans. It has a major historical significance making it a globally popular beverage (Weisburger, 1997). Tea was first utilized as herbal medicine in the Yunnan province of South Western China(Pan et al., 2022) . Due to its distinct aroma and flavour, tea became increasingly trendy as a leisure and therapeutic beverage in China, and it was introduced and spread to western parts of the world during the sixteenth century(Gilbert, 2008). Global consumption of tea equals the sum of all other drinks combined, which include soft drinks, coffee, and alcoholic beverages (Pan et al., 2022). Tea has been shown to have a numerous health benefits and roles in the human body (Gilbert, 2008; Karak et al., 2017). Research has also shown that it has antimicrobial properties (Chen et al., 2019). In terms of culture, market, function, and taste, no other beverage can compete with tea.

Processed tea from *Camellia sinensis* is classified in to one of four categories based on the degree of oxidation and the method of processing. These include: - non-aerated tea such as

green or purple tea which are or is the least oxidized, semi-oxidized tea such as white and oolong tea as well as black tea which has a degree of oxidation of 100 % and post fermented tea which in addition to oxidation have microbial fermentation(Ochanda and Ruto, 2022).

Recent research has focused on the chemical composition of tea, including phenolic substances (Sang et al., 2011), nonetheless, few research works on the microbial component of tea as well as its health effects have been conducted. As a result, the purpose of this research is to examine the microbial profile of various types of tea (black tea and green teas). Seeing as tea is typically prepared by brewing in hot water (approximately 90 °C) and sometimes boiling water (100 °C), this procedure is capable of killing a vast number of microbes (Brock, 1985). The study will also looked into the effect of household tea-making techniques (infusing with hot water) on tea's microbial profile.

2.2.2 Important Components of Tea

Tea is composed of a variety of compounds, which include polyphenols, alkaloids (Graham, 1992), proanthocyanidins, amino acids (Essential Amino Acids: Leucine, Phenyloalamine, Valine, and Threonine), L-theanine, carbohydrates, Vitamins (Tocopherol, the B complex; Riboflavin, Biotin, Niacin, Pantothenate, Inositol), chlorophyll, minerals, trace elements as well as unidentified components (Wiseman et al., 1997). The amino acids in tea are responsible for brothy or umami flavour. Theanine is the most abundant amino acid in made teas. L-Theanine in particular, promotes brain relaxation and when coupled with caffeine, may also induce a state of "mindful alertness" (Boros et al., 2016).

Polyphenols are the most studied and the primary bioactive molecules found in tea (Cabrera et al., 2003). The most abundant form of polyphenols in tea is flavan-3-ols and catechins (Balentine et al., 1997). Theaflavins and Thearubigins are the oxidated form of flavan-3-ols and are at high levels in made black tea. The other classes of the flavonoids include the flavonols (quercetin, myricetin and kaempferol) and flavones (Wiseman et al., 1997).

Tea naturally produces two types of enzymes, that is, polyphenol oxidase (PPO) and polyphenol peroxidase (POD) that catalyze oxidation of polyphenols. Oxidation occurs after maceration thus exposing polyphenols to oxygen leading to the enzymatic browning of tea leaves (Manzocco et al., 1998). For the processing of green tea, heat could be used to denature or deactivate the enzymes, preventing browning as first step in the green tea production. Enzymes can also be denatured by completely eliminating moisture from them for an extended period of time, as occurs during the lengthy withering duration in white tea manufacture (Chen et al., 2019).

In addition, tea has chlorophyll and carotenoid pigments that absorb light utilized in photosynthesis. Withering and oxidation in the production of black tea cause them to condense and darken and in the process, tea chlorophyll turns black as oxidation progresses (Manzocco et al., 1998). Carbohydrates in the aid in the formation of polyphenols in tea plants and promote enzymatic reactions as oxidation occurs in black tea processing. There are volatile substances present in tea leaves responsible for the made tea’s aroma and savour (Ho et al., 2015). Tea's aroma complex is composed of a variety of flavour and aroma compounds which occur in small quantities. Most of such aromatic compounds are

synthesized during the processing of tea leaves and do not exist in fresh tea leaves (Ho et al., 2015).

2.2.3 Health Benefits of Tea

Consumption of tea has been linked to a variety of health benefits, which could be due to its high range of bioactive molecules (such as polyphenols), that have been shown to have antioxidant and antiviral properties, as well as the ability to alleviate inflammatory conditions (Karori et al., 2008), modifies the activity of detoxification enzymes like glutathione peroxidase and glutathione reductase (Mandel et al., 2006). Polyphenols and other antioxidant compounds found in tea aid in the improvement of immune function and minimizing the aggregation of platelets (Lampe, 2003; Frankel and Finley, 2008). Consumption of green tea (GT) intake, has also been linked to a lower prevalence of chronic illnesses associated with oxidative stress, including cancer (Butt and Sultan, 2009; Chung et al., 2003) and cardiovascular diseases (CVDs); (Stangl et al., 2007; Babu and Liu, 2008). The tea polyphenols act synergistically with ascorbic acid to strengthen the capillary blood vessels and also exhibit an anti-atherosclerosis action. Polyphenols in tea, particularly those with the galloyl moiety, have been shown to block HIV entry by binding to gp41, which is required for HIV-1 binding to the cells (Shuwen et al., 2005). Green tea polyphenols were discovered to be more effective than black tea polyphenols in inhibiting the growth of CAL-27 cells in a dose-dependent fashion. In addition, the mixture of green tea polyphenols and b Lactoferrin (bLF) in a ratio of 1:2 inhibited CAL-27 cells synergistically (Chandra et al., 2007).

The green tea's health-promoting properties are mostly due to its polyphenol element known as catechins. Epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epicatechin (EC) are the four major forms of catechins. Green tea polyphenols have been shown to inhibit the growth of pathogenic bacteria like *Helicobacter pylori, Vibrio cholerae, Streptococcus sobrinus, Salmonella typhi,* methicillin-resistant *Streptococcus mutans Shigella dysentery, Shigella flexneri,* and *Staphylococcus aureus* (Bharadwaj et al., 2022). Green tea polyphenols have also been shown to be active against the human immunodeficiency virus (HIV), hepatitis and influenza viruses (Li et al., 2011). Epigallocatechin gallate (EGCG), a form of tea polyphenol, has been found to be responsible for most of GT's health-promoting properties (Khan et al., 2006).

Generally, due to the greater concentration of EGCG in GT, it has been proven to be effective compared to black tea (BT) in terms of health benefits, though the function of thearubigins (TRs) and theaflavins (TFs) in BT has not been thoroughly examined. In vitro studies and animal models demonstrate that polyphenols extracted from green tea have bioactivity to postpone the initiation of disease-related risk factors (Cabrera et al., 2006; Yang et al., 2007).

According to a report published in the *Archives of Internal Medicine* in July, 2004, drinking tea regularly for at least a year lowered the risk of developing high blood pressure (46 % for those who drank 0.5 to 2.5 cups per day and 65% lower for others who drank more than 2.5 cups per day). Daily intake of tea has also been related to UV protection, skin structure and function maintenance (Heinrich et al., 2011) and it has also been shown to reduce the

risk of cognitive decline and to mitigate or stop the progression of dementia (Ng et al., 2008; Chen et al., 2019).

Tea polyphenols' ability to scavenge free radicals is attributed to the presence of a phenolic hydroxyl group bonded to the flavan-3-ol structure, which has been linked to teas' therapeutic effect against free radical-mediated diseases, generating a lot of interest in science (Amie et al., 2003). Environmental contaminants, radiation, chemicals, toxins, physical stress, and the degradation of drugs and food all contribute to the development of free radicals. Free radicals are linked to the pathogenesis of chronic and degenerative diseases in humans, such as cancer, gastritis, Central Nervous System (CNS) injury, atherosclerosis, arthritis, Parkinson's disease, Alzheimer's disease, Acquired Immune Deficiency Syndrome (AIDS), diabetes, dementia, ischemia and renal disorders, (Pourmorad et al., 2006; Rao et al., 2006). The fluoride in tea prevents tooth decay by stopping pathogenic and commensal microorganisms from demineralizing the enamel (protective coat) (Hamilton-Miller, 2001).

2.2.4 Different Types of Made Teas and Their Processing Methods

2.2.4.1 Black/Aerated Tea Processing

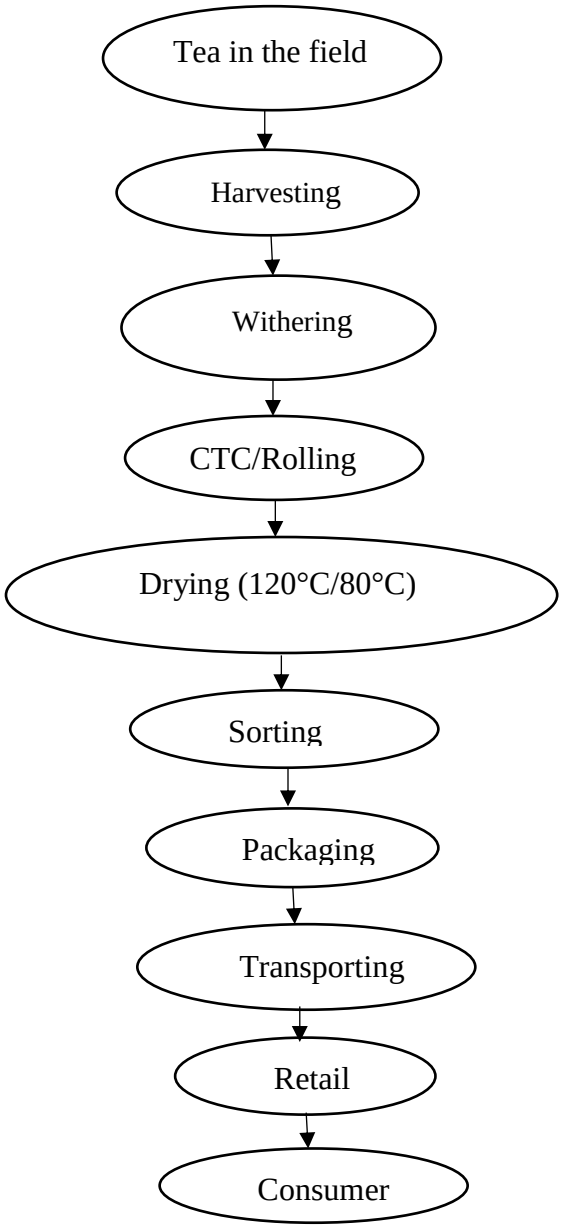

Figure 2.1: Black CTC tea production flow diagram.

Aerated tea is be processed by withering for a period of between 16-18h. Leaves are macerated using a Cut Tear Curl (CTC) machine. The crushed leaf is then allowed to undergo oxidation for about 90 mins with the help of enzymes naturally present in the tea leaves, this is a crucial step in the production of astringent taste and aroma compounds in black tea, as well as the colour, weight, and briskness of the liquor. The tea is dried to moisture content 5 % (Temple et al., 2000).

2.2.4.2 Green Tea Processing

Fresh leaves are harvested from the *Camellia sinensis* plant and immediately are heat treated by steaming or panfrying to inactivate enzymes. The teas are then withered and then macerated either by CTC or orthodox method followed by drying (Ahmed and Stepp, 2013).

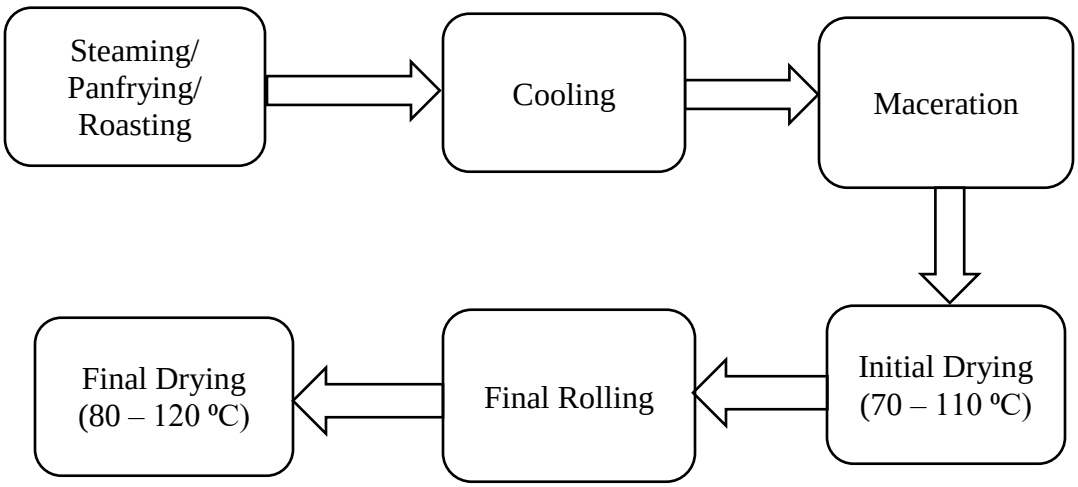

Figure 2.2: Green tea processing

Before the leaves are rolled into form, light heat is applied to them by steaming or roasting. This helps to stop the oxidation process. The fresh, grassy flavour of the leaf is enhanced

by steaming. After rolling, green tea leaves are not permitted to oxidize, and this is why they retain their light colour as well as flavor (Guang et al., 2007). The leaves are rolled after steaming and withering, then spread dried. Green tea liquor is typically green in colour, with flavours ranging from grassy (pan fried teas) to toasty to fresh steamed greens (steamed teas) which are mostly mild and have astringency that is vegetable-like (Balentine et al., 1997).

2.2.4.3 Purple Tea Processing

TRFK 306 is a new purple tea coloured variety or clone released by KALRO-Tea Research Institute (Formerly Tea Research Foundation of Kenya) (Kamunya et al., 2009). Purple tea was developed specifically for tea health products because of its high antioxidant capacity, making it more therapeutic than green and black teas. It contains a flavonoid called anthocyanin, which gives the leaves a purple colour (Kerio et al., 2011). Purple tea is processed the same way as green tea.

2.2.4.4 Yellow Tea Processing

This is normally processed like green tea, but rather than drying immediately after fixation and maceration, the leaves are heaped, covered, in a humid environment, a process known as sweltering. Chlorophyll is oxidized, yielding a yellowish tea (Zhou et al., 2005). The tea is thereafter dried and packaged. Sweltering induces chemical changes in polyphenols and amino acids in the processed tea leaves, giving it a distinct brisk and mellow flavour (Varman and Sutherland, 1994).

2.2.4.5 White Tea Processing

This is a rare specialty type of tea whose uniqueness comes from the particular portion of leaf and post-harvest treatment that the leaves are subjected to. The white tea leaves include the buds or the buds and one leaf and are collected and dried. This tea undergoes minimal amount of processing (Ochanda and Ruto, 2022).White tea contains the highest quantity of polyphenols and has the lowest yield rate the unit area in terms harvested leaf used as raw material (Dias et al., 2013).

2.2.4.6 Oolong Tea Processing

Oolong tea is made by withering the leaves to a moisture content of about 60 % then partially fermenting the leaves, that is, shorter fermentation period than black tea this may range from 25 to 75 %. This is then followed by firing. The result is a product that has characteristics falling between green and black teas. Its colour, taste and aroma are delicate compared to black tea but richer than those of green tea and it is usually greenish-brown in colour (Duthie and Crozier, 2003; Del Rio et al., 2004).

2.2.5 Tea Standards

Made tea should meet certain physical, chemical and microbial requirements as stipulated both nationally, regionally and even internationally without which, the tea cannot qualify for export. For hygiene purposes, black tea shall be processed, manufactured and handled in a hygienic manner in accordance with East African Standards EAS 39.

Food safety is an important component of food for human consumption. Microbial contamination in foods may spoilage and diseases to consumers and is of great concern as it endangers public health (Gizaw, 2019). The made teas have been found to be contaminated with yeast and moulds which usually cause spoilage. Their allowable quantity is given as colony forming units per gram (CFU/g) and should not exceed 10^3 (ISO 21527-2). The disease causing microorganisms include *Staphylococcus aureus* (ISO 6888-1), *Escherichia coli* (*E. coli*) (ISO 7251) and *Salmonella spp* which should all be absent.

2.2.6 Bacteria in Tea

Bacteria from the *Bacillaceae, Staphylococcaceae,* and *Paenibacillacea* families, which are part of the skin flora while others are soil-dwelling, have earlier been detected in processed tea samples (Tan et al., 2016). Only black tea, which has been fermented and thoroughly oxidized, was shown to contain the greatest bacterial counts and the most diversified bacterial composition (Tan et al., 2016). Post fermented tea has been found to contain *Bacillus coagulans,* a probiotic bacterium that is beneficial to the human body. This bacterium was identified after isolates from these teas were analyzed using Random Amplified Polymorphic DNA (RAPD) method.

It has been observed that catechins, which are abundant in green tea, have no effect on the growth of certain health-promoting bacteria like *Eubacterium rectale*, *Bifidobacterium spp* and *Lactobacillus spp* (Xin et al., 2013). The pathogenic fungi *Cryptococcus neoformans* and *Candida albicans* have been observed to be inhibited by theaflavin- 3, 3'- digillate (Koech, et al., 2014). Catechins, theaflavins, and thearubigins, among other tea

polyphenols, have been shown to have synergistic antimicrobial effect against some infections (Koech, et al., 2014). Bacteria from the *Staphylococcaceae* and *Paenibacillacea* families have been identified in post-fermented teas in previous research (Tan et al., 2016).

2.2.6.1 Escherichia coli

It is a facultative anaerobic gram-negative rod-shaped bacterium. Theodor Escherich was the first to describe it in 1885. The majority of *E. coli* strains are safe bacteria that colonize the gastrointestinal tracts of people and animals as part of normal flora. Nonetheless, some strains of *E. coli* have developed into pathogenic *E. coli* as a result of virulence factors acquired via plasmids, transposons, bacteriophages, and pathogenicity islands. Serogroups, clinical symptoms, pathogenicity mechanisms and virulence factors can all be used to classify pathogenic *E. coli* (Kaper et al., 2004). Enterohemorrhagic *E. coli* (EHEC) is a kind of pathogenic *E. coli* that causes haemorrhagic colitis (HC) and the potentially fatal sequelae haemolytic uremic syndrome (HUS) among people. Numerous EHEC serotypes including:-O26:H11, O91:H21, O111:H8, O157: NM, and O157:H7, are commonly linked to human illness (Paton et al., 1999; Melton et al., 1996).

2.2.6.2 *Salmonella* spp.

These are gram-negative, facultative anaerobic bacteria that are rod-shaped and belong to the Enterobacteriaceae family. The intestinal tracts of animals and humans are their primary habitat. A few species exist in animals without exhibiting clinical signs; others can cause a variety of moderate to severe diseases in people known as salmonellosis. The

majority of *Salmonella* infections in humans are caused by ingesting contaminated food or drink (Linam and Gerber, 2007).

Typhoid fever is caused by *Salmonella typhi. S. paratyphi, S. schottmuelleri, and S. hirschfeldii* are variants of *S. enteritidis* that cause paratyphoid fever. Refrigeration slows bacterial replication but does not kill them so various *Salmonella spp.* can grow in foods, causing gastroenteritis if consumed (Ehuwa et al., 2021).

2.2.6.3 Staphylococci aureus

This round-shaped bacterium belongs to the *Firmicutes* family and is Gram-positive. It is a common microbe found in the body particularly on the skin and in the upper respiratory tract. It is a facultative anaerobe that can develop without oxygen and tests positive for nitrate reduction as well as catalase (Masalha et al., 2001). Even though *Staphylococcus aureus* (*S. aureus*) is normally found in the human microbiota as a commensal, it could also act as an opportunistic pathogen, causing skin infections like abscesses, respiratory infections including sinusitis and food poisoning. Infections are also aided by pathogenic strains developing virulence factors like potent protein toxins as well as the production of a cell-surface protein which binds antibodies thus inactivating them. Antibiotic-resistant *S. aureus* strains, including methicillin-resistant *S. aureus* (MRSA), have emerged as a global clinical concern (Schlecht et al., 2015). *S. aureus* vaccines have yet to be accepted, despite extensive research and development.

Long-term carriers of *S. aureus* are believed to account for 20 % to 30 % of the human population (Tong et al., 2015), which is present in the lower reproductive tract of females,

as part of the natural skin flora, in the nostrils (Cole et al., 2001) and also as part of the natural skin flora (Hoffman, 2012). From mild skin conditions like boils, pimples, cellulitis, folliculitis, abscesses, scalded skin syndrome and carbuncles, to life-threatening fatal infections like pneumonia, toxic shock syndrome, meningitis, bacteraemia, osteomyelitis, endocarditis and sepsis, *S. aureus* may lead to a number of ailments. It remains among the top five main sources of hospital-acquired bacterial infections, and it's a common source of bacterial infection after surgery (Schlecht et al., 2015).

2.2.7 Fungi in Tea

Contamination of tea by fungi may take place at the different stages of processing. Different types of tea can be invaded by various mycoflora. Wet and warm climate encourages growth of fungi which are the same conditions that are usually also favourable for tea cultivation. *Fusarium* is the primary genus detected in the soils of China's tea plantations in the subtropics (Wang et al., 2018). Mycoflora present in the field is responsible for production of mycotoxin including: fumonisins, T-2, HT-2 toxins and deoxynivalenol as well as its derivatives (Sedova et al., 2018). Handling processes that follow storage together with consumer delivery may also lead to microbial contamination of made teas.

Green tea takes the shortest time to process where, fresh leaves collected from the farm are first steamed or panned immediately to stop oxidation therefore polyphenols are retained in their original state. After being steamed, the tea is then rolled and then dried. During processing, green tea is therefore expected to have the least levels of microbial

contamination. Black tea production takes a longer period than green tea. The additional steps involved in black tea production include wilting and fermentation which result in polyphenols found in tea being condensed to form thearubigins and theaflavins through oxidation.

Storage fungi are significant contributors to tea degradation. Studies have shown that numerous contaminations occur at the beginning of tea processing (Sedova et al., 2018). This was due to the fact that isolated fungi are widely known airborne contaminants with a high propensity for proliferation, mostly when food for growth can be supplied by the leave remnant. Tea spoilage is mostly followed by mycotoxin production by certain tea-borne fungi as secondary metabolites and they are usually toxic (Khosravi et al., 2012). *Aspergillus flavus* and *A. parasiticus* are groups of fungi that produce a type of mycotoxins known as Aflatoxin both on the growing tea plant in the field and even during the storage of made tea. According to research, aflatoxins are highly poisonous, cancer causing, mutagenic and also teratogenic (Seeshti et al., 2013). Extensive studies on fungi as well as mycotoxins found in seeds, livestock feed and human diets have been conducted (Marin et al., 2013).

The purpose of this research is therefore to determine the impact of handling during processing on mycoflora profile of Kenyan black and green teas. Furthermore, if *Aspergillus flavus* is present in the teas, production of aflatoxin on different types of tea will be determined. Specific fungi that can occur on a range of crops produce mycotoxins, which are abiotic poisons (Marin et al., 2013). Tea mycotoxin generation can happen at any point during the manufacturing process, including tea bush cultivation, harvesting or

collection, processing in the factory and even during storage. Poor farming techniques, as well as inappropriate processing, drying, packaging, storage, and transportation conditions, encourage fungal development, increasing the risk of contamination with mycotoxin. Subtropical climate, which is ideal for tea growing, is also optimal for the development of toxicogenic mould. Tea samples were shown to be contaminated with *fumonisins*, *deoxynivalenol*, and *enniatins* in recent concerns targeted at multi-mycotoxin analyses *(Sedova* et al., 2018). The present study aims to identify and characterize microbial quality of green and black, teas.

2.2.7.1 Yeast

Yeasts could have adverse effects on fermented foods ingested by animals and humans in both positive and negative ways. They are employed as starter cultures in cheeses and breads, as well as wine, beer, and other alcoholic fermented beverages (Maicas, 2020). Yeast can, nonetheless, cause food and beverage deterioration. The fact that yeasts have been identified from a variety of rotting foods and beverages demonstrates this. In a variety of settings, yeasts contribute to spoilage; therefore several biological and chemical techniques have been used to inhibit their multiplication (Lowes et al., 2000; Rawat, 2015).

2.2.7.2 Moulds

Moulds that can contaminate tea during production and manufacturing include: *Aspergillus*, *Penicillium*, *Pacelomyces*, *Cladosporium*, *Alternaria*, *Mucor*, *Fusarium*, *Rhizopus*, *Absidia* and *Trichoderma* species (Halt, 1998; Elshafie et al., 1999; Martins et al., 2001; Romagnoli et al., 2007; Mogensen et al., 2009). Toxigenic moulds including

Aspergillus, *Penicillium*, *Fusarium*, and *Alternaria* species generate harmful secondary metabolites widely recognized as mycotoxins. Humans are exposed primarily through ingestion of mycotoxin-contaminated food items, which causes serious medical complications such as immunosuppression, carcinogenesis, along with genotoxic, hepatotoxic, as well as nephrotoxic implications (Santos et al., 2009; Milićević et al., 2010; Monbaliu et al., 2010).

2.2.7.3 Mycotoxins Found in Tea

For the past few decades, researchers have investigated the impact of tea intake on health and nutrition. Interestingly, little thought was given to its safety. Pesticides, heavy metals, polycyclic aromatics, microbes, radionuclides, plant growth regulators, and mycotoxins were all addressed in Abdel and El-Maghraby's review of tea contamination. The researchers reported that contaminants leached into the tea liquor were either undetected or were below the permissible levels. However, recent research has shown that mycotoxins in tea should be given greater attention (Abdel and El-Maghraby, 1992)

2.2.7.4 Mycotoxin Transfer from Raw Tea into the Beverage

Raw tea contamination, mycotoxin heat stability, and mycotoxin capacity to transfer from matrix into aqueous infusions are factors that could affect mycotoxin concentrations in tea liquors. Common mycotoxins cannot be substantially degraded during brewing. Water can extract numerous water-soluble mycotoxins from it's the tea matrix, including aflatoxins (10–20 mg/mL), fumonisins (approximately 20 mg/mL), zearalenone (0.02 µg/mL),

ochratoxin A (0.0004 mg/mL as acid, water-soluble as salt), T-2 toxin (approximately 0.1 mg/mL), and deoxynivalenol (55 mg/mL) (Sedova et al., 2018).

In this context, the transmission of mycotoxin from naturally contaminated made tea to beverage infusion was investigated in the sampled tea. Aflatoxin transmission was reported by Monbaliu et al., 2010 to be 28–33 % in experimentally contaminated made tea samples. Upon brewing a naturally contaminated made tea sample (76 g/kg), Monbaliu et al., 2010 were unable to detect fumonisin B1 in infusions despite fumonisins' high water solubility, which they ascribed to the method's limited sensitivity. Tea made from tea bags contained aflatoxins and 15-acetyl deoxynivalenol, according to a multi-mycotoxin investigation (Sedova et al., 2018).

2.2.7.5 Exposure Assessment and Legislation

Aflatoxin B1 and ochratoxin A are the most lethal mycotoxins. There are ppb maximum levels of these mycotoxins set for food commodities. The potential for dietary exposure to these pollutants as a result of tea intake will be assessed. Mycotoxins in tea are almost completely unregulated in some countries. In Customs Union nations such as Armenia, Belarus, Kazakhstan, Kyrgyzstan, and Russia, however, national legislation governing mycotoxins in tea have been adopted. An example is Argentina which set a limit for aflatoxin B1 and total aflatoxins in ingredients for use in herbal tea infusions at 5 and 20 µg/kg, respectively and that for aflatoxin B1 in raw tea at 5 µg/kg (Sedova et al., 2018). In Asian countries, upper limits for the category "all foods" have been set at 30 µg/kg for aflatoxin B1. In India, it is set at 30 µg/kg for total aflatoxins. Aflatoxin limits in Japan are

set at 10 µg/kg while in china it is 5–20 µg/kg. All these limits are dependent on the food matrix (Sedova et al., 2018). Due to a lack of occurrence data, it is impossible to conclude that public health protection measures are necessary. In order to obtain controlled mycotoxins levels in Kenyan teas, representative studies of various types of tea must be conducted.

2.2.8 Quality Control in the Tea Industry

Kenyan tea industry standards are based on the International Organization for Standardization (ISO) and the Codex Alimentarius Commission (CAC) (Oloo, 2011) standards of trade. Throughout the food supply chain, farmers must practise Good Agricultural Practices (GAPs), Good distribution practices must be followed by local and foreign sellers of commodities/raw materials, and Good Manufacturing Practices must be followed by producers (GMPs). Operators in the food supply chain must follow either national (mandatory) and or private (voluntary) requirements. Chain supporters offer the required propulsion, whereas chain enablers offer the necessary control and/or regulation. The Kenya Bureau of Standards serves as the primary chain enabler and point of contact for the national codex (Oloo, 2011). The Tea Directorate conducts ongoing enforcement audits of tea factories on tea laws, efficiency, and guidelines, as well as aspects of good agricultural practices (GAPs), good manufacturing practices (GMPs), and best practices, in needed to guarantee the continued protection and quality for both the domestic and foreign markets. The following are some of the most important national legislation areas for quality and safety compliance: Environmental Management and Coordination Act 1999 on production, processing and handling of tea; Occupational Safety and Health Act

(OSHA), 2007 that is a work place registration certificate; The Food, Drug and Chemical Substances (Food Hygiene) Regulations (Cap 254) meant for the tea factory and factory employees; Kenya Standard-KS 459, a standard for portable water; Kenya Standard-KS 40 ,standard for pre-packaged product labelling; Kenya Standard-KS 1927, set of requirements that must be met for Tea packets and containers; Kenya Standard-KS 1972, a protection, consistency, and integrity standard for bulk tea packaging; Kenya Standard-KS65 requirements for black tea quality. In addition, factories are encouraged to obtain ISO certification in Quality Management Systems (ISO 9001:2008), Food Safety Management Systems (ISO 22000), and Environmental Management Systems (ISO 14001:2004).

2.3 Theoretical Frame Work

The theoretical framework for the study titled "Characterization of microorganisms in made black cut tear and curl (CTC) and green orthodox tea along the tea value chain from selected Kenyan tea factories" draws upon various theoretical perspectives and concepts from the fields of microbiology, food science, chemistry, and biochemical science. This framework provides the theoretical underpinning for the research and guides the investigation into the quantitative and qualitative microbial composition and quality assessment of made black and green teas from selected tea factories in Kenya.

Microbiological principles and techniques: The study is anchored in understanding microbial communities in processed conventional and specialty tea products, including factors affecting their growth, survival, and interactions. The research study utilizes appropriate methods for detecting and quantifying microorganisms in the sampled

processed black and green Kenyan teas. The various microbiological techniques aid in microbial enumeration, culture-based identification and characterization (Jany and Barbier, 2008).

Ecological systems theory**:** This theory suggests that the microbial composition of tea was influenced by a complex interaction of environmental factors including soil microbiota, climate, and altitude and also human activities that comprised of processing methods. The research study considered how these factors interacted within the tea production value chain across the different tea growing regions in Kenya (Prosser et al., 2007).

Food safety, quality assurance and control: The importance of quality assurance and control measures throughout the tea production value chain to mitigate microbial contamination was highlighted in this research study. This involved good agricultural practices (GAPs), hygienic processing conditions and effective sanitation practices (WHO, 2018). The importance of monitoring microbial contaminants to ensure compliance with food safety standards including microbial limits and hygiene practices was also looked into (FDA, 2020). Principles of Hazard analysis critical control points (HACCPs) and its application in ensuring food safety, including its relevance to the tea industry were applied. This ensured that potential hazards in tea processing such as raw material handling and processing equipment that could lead to microbial contamination were identified .Furthermore quality assurance protocols in tea processing to maintain product consistency and consumer safety was incorporated in the study.

Regulatory tea standards: This study widely examined relevant local regional and international standards for microbial contamination in tea. These standards included those set locally by the Kenya Bureau of Standards (KEBS), regionally, that is, the East Africa

Standards (EAS) and internationally by International Organization for Standardization (ISO) and the Codex Alimentarius Commission (CAC) to regulate the levels of microbial contaminants permitted in made conventional and speciality teas (Codex Alimentarius Commission, 2019; ISO, 2017).

Microbial contaminants in tea Processing and factors affecting their growth: This research study was aimed at understanding the types of microbial contaminants that could affect tea during processing. The target microbes included bacteria (*E. coli, Salmonella spp.* and *Staphylococcus aureus*) and fungi (yeast and molds). There was also identification of factors that influence microbial growth in Kenyan made tea, such as moisture content, temperature during drying process, handling and the ultimate packaging operations.

Cultural determinism: The research study explored how geographic and cultural factors specific to each region influence the microbial profile of tea. Different regions in Kenya may vary in terms of microbial contamination due to climate differences, soil composition, and tea Processing and tea brewing techniques. Profiling for regional variations in microbial species diversity and abundance in processed tea was carried out (Maron et al., 2011).

Emerging issues and research gaps: This study was also aimed at identifying current research gaps and emerging issues in microbial assessment of Kenyan made conventional and speciality teas. This included the impact of climate change on microbial profiles and the efficacy of novel processing technologies in reducing microbial contamination.

By drawing on these theoretical perspectives and concepts, the research study aims to comprehensively characterize microorganisms in made black cut tear and curl (CTC) and

green orthodox tea along the tea value chain from selected Kenyan tea factories. This theoretical framework provides a structured approach to conducting a scientific research study on the microbial composition of processed Kenyan tea, integrating microbiological, ecological, and quality control principles to address important issues in food safety and quality assurance. This multidisciplinary approach ensures a holistic understanding of the factors influencing tea microbial quality and its broader implications. This would equip the tea industry stakeholders and give recommendations for future research to further enhance tea quality and safety.

2.4 Identification of Knowledge Gap

There was a gap in research regarding the actual exposure of consumers to microbial contaminants through consumption of made black CTC and green orthodox teas from different regions in Kenya. More studies were needed to assess consumer exposure levels and associated health risks linked to microbial contamination in tea. This information would help inform risk assessment and mitigation strategies to protect consumer health.

This study also sought to find the effectiveness of set standards, regulations and control measures that have been put place in Kenyan tea factories. Even though there are set guidelines and standards for microbial control in tea processing like HACCP and KEBS standards empirical data on the effectiveness of these control measures in various Kenyan tea factories is limited. This research study sought to evaluate the implementation and efficacy of the control measures at critical control points. This was aimed at ensuring that they effectively reduce microbial load in Kenyan made tea products.

Research on the impact of processing techniques on microbial contamination of Kenyan made black CTC and green orthodox teas is limited. Variations in processing methods, such as drying, fermentation, and storage conditions, could significantly affect microbial growth and survival. Understanding how these techniques influence microbial populations in tea is crucial for optimizing processing practices to ensure food safety and quality.

Previous research on microbial assessment in Kenyan made teas lacked comprehensive studies that encompass multiple factories in the various tea-growing regions. There was need for studies that systematically compared microbial profiles and contamination levels across the Kenyan factories in the different regions putting into consideration their different processing techniques and adherence to food safety measures.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter describes the study area, research design, sample collection methods and the data analysis techniques that were used in this study.

3.2 Research Design

An experimental research design was used for this study.

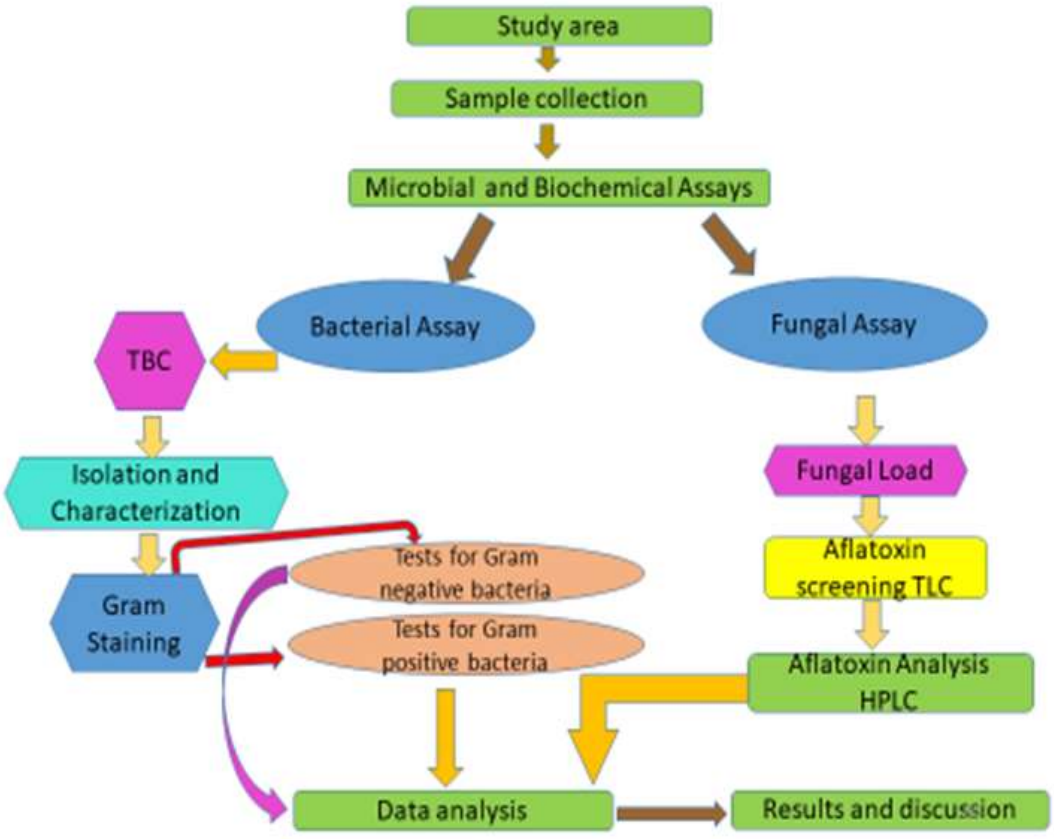

Figure 3.1: Experimental research design used in the research study.

Desktop research and data collection on the performance of Kenyan tea factories was done and factories in the east and west of the Kenyan Rift Valley were selected for the study. The best and least performing factories were purposely selected from the Kenya Tea Development Agencies zonal demarcations of Kenya's tea growing regions in the East and West regions of the Rift valley. Those factories had the highest and lowest prices at the Mombasa auction in the year 2018. Kenya's tea growing regions have unique soil, rainfall and climate which differ in altitudes. Different tea cultivars are grown in these areas and these cultivars have unique biochemical profiles, aromas and produce tea with unique liquors, aroma and taste.

3.3 Location of Study /Site

Tea is grown over the highlands East and West of the Rift Valley regions in Kenya which are cooler and wetter compared to other non-tea cultivating regions. These regions are within the altitude range of 1500-1700 m above sea level and with red volcanic soils conditions that are favourable for tea production. The tea growing regions cover area of Latitude 1.3N to 1.3S and Longitude 38.4E to 34.3E as shown on the map in appendix 3.

Tea being a highland crop grows well where the soils have proper drainage such as in the sloppy terrains. These areas are located mainly in the Kenyan highlands and include Kericho, Nandi, Kakamega, Kisii and Cherangani hills in the Highlands make up the West of the Rift Valley region. On the other hand, Nyambene hills, Nyeri, Murang'a, Thika and Maragua in the Highlands make up the East of Rift Valley region. In total there are seven tea growing regions in Kenya according to KTDA as shown on the map in appendix 2.

3.4 Sample and Sampling Procedures

Fresh leaf, in process tea, made tea, surfaces, equipment and material were aseptically collected from selected Kenyan tea factories which included Kenya Tea Development Agency managed factories, a multinational and a private factory.

In determining the tea quality along the value chain a multinational and a private factory were selected. Tea samples were collected along the whole value chain from raw materials, in process to finished product and were used for analysis of microbial quality. Fresh leaf was collected from the reception area for swabbing and subsequent microbial analysis. In process tea from withering, maceration and fermentation just before the drying stage were also collected. The finished product was collected just after drying, at sorting and grading and storage in the storage bins and after packaging in various packaging containers.

In the determination of quality for processed teas, samples consisted of all the tea grades from the drier mouth and bins in a total of 14 pre-selected factories from the East and West of Rift Valley tea growing regions of the KTDA managed factories.

Processed and aseptically packaged teas were collected from factories in nine (9) tea growing counties -Kericho, Kiambu, Trans Nzoia, Kirinyaga, Narok, Nyamira, Meru, Nandi and Elgeyo Marakwet. The processed teas were collected using simple random method of sampling. Samples were collected from one factory from each of these counties, making a total of 15 factories from the East and West of Rift regions. Two hundred and fifty grams (250 g) of each tea grade (BP1, PF1, PD, D1 Fannings, and D2) was collected from each factory in duplicate for purposes of reproducibility and placed in aluminium bags. A total of 126 black CTC tea samples were collected. They were then packed in zip

lock bags to avoid contact with moisture during transportation in cooler boxes to the Tea Research Foundation Miniature Factory for storage at room temperatures (22-24 ºC) and Relative humidity awaiting analysis.

3.5 Data Collections Instruments

Various data collection instruments and tools were used in the characterization of microbes in Kenyan made black CTC and green orthodox teas from the East and West of Rift Valley regions to gather comprehensive data on microbial contamination.

Sampling tools and equipment that were used included sterile sampling aluminum lined bags placed in sterile cool boxes, sampling scoops and appropriate personal protective equipment (PPE) to minimize cross-contamination during sample collection.

Laboratory equipment which comprised of a biosafety cabinet, where inoculation and isolation was done, an incubator used to cultivate microorganisms under controlled conditions to promote their growth for enumeration and identification. An autoclave was used for sterilization of media, containers and instruments to ensure aseptic conditions in the laboratory. A microscope was used for visual inspection of microbial colonies and morphology during microbial identification.

The microbial analysis tools used were culture media used to culture and isolate different types of bacteria and fungi present in tea samples. Petri dishes were used for streaking samples onto agar plates for microbial colony growth and enumeration. A colony counter was used to count microbial colonies on agar plates accurately.

The data recording and analysis tools employed included a laboratory notebook for recording detailed notes and observations during sample processing and analysis. Excel software was utilized for organizing and managing data collected during sampling and laboratory analysis. Whereas statistical analysis software (SAS) was used for analyzing data on microbial counts (colony forming units), identifying trends and comparing microbial contamination levels among factories in the two tea growing regions in Kenya.

The quality assurance tools employed comprised of positive and negative controls as well as sterility check. The documentation and reporting tools used included standard operating procedures (SOPS) for sample collection, laboratory analysis and data management which ensured consistency and reproducibility of results. Report templates were used for summarizing findings, including tables, graphs and interpretations of microbial contamination levels in Kenyan made black CTC and green orthodox teas from both regions.

3.5.1 Validity

The validity of data collection instruments used was done by ensuring that they accurately measured what it was intended to measure. Construct validity to check that the instruments aligned with theoretical concepts in this research study through literature review and expert evaluation was ensured. Content validity was also checked so that the instruments comprehensively covered all relevant aspects of this research study on characterization of microbes in Kenyan made black CTC and green teas from selected factories.

3.5.2 Reliability

The consistency and stability of data collection measurements collected using the instrument over time and across different conditions in microbiology was ensured during this research study. This was achieved through replication, in that all the laboratory analysis was done in triplicates to ensure in consistency and reproducibility of results collected using the data collection instruments.

3.6 Microbial and Biochemical Assays

3.6.1 Bacterial Assay

3.6.1.1 Total Bacterial Count

Total bacterial count (TBC) analysis, was carried out according to International Organization for Standardization (ISO) Procedure (Carraturo et al., 2018; ISO 4833-1:2013). Plate Count Agar (PCA) and Nutrient agar (NA) were prepared, sterilized and poured into sterilized petri dishes to solidify. Approximately 100 µL of the sample was pipetted onto PCA for standard plate count. It was then spread on the entire surface with using a sterilized bent glass rod. This was done in three replicates. The PCA plates were then incubated in an inverted position at 37 °C for 24 h. A colony counter was used to count all of the colonies and the results were recorded (Qazi et al., 2008). Different isolates were sub-cultured in fresh nutrient agar so as to undergo further microbial and biochemical analysis for identification. They were processed for the determination of Gram's reaction and cell morphology, motility, catalase and oxidase tests (Carraturo et al., 2018)

3.6.1.2 Isolation and Characterization of Bacteria

This was done by weighing 0.5 g of the tea samples and infusing with 10 ml of distilled water at room temperatures (22-24 ºC). One hundred (100) µL of the tea samples was then inoculated in Nutrient agar at 37 ºC for 24 h for bacterial growth. Sterilized distilled water was used as a negative control.

3.6.1.3 Gram Staining for Identification of Bacterial Isolates

An air-dried heat-fixed cell smear was flooded with crystal violet staining reagent for 1 min. The slide was then washed for 2 s in a gentle, indirect stream of tap water. Thereafter, it was filled with Gram's iodine, which is a mordant, and left for 1 min for the stain to fix. The slide was then washed in a gentle and indirect stream of tap water for 2 s before flooding it with a decolorizing agent (absolute ethanol) and allowed to stand for 15 s. Afterwards, safranin dye was flooded onto the slide for 1 min to counter-stain the microbes. Finally, the slide was washed in a gentle and indirect stream of tap water until no colour appeared in the effluent. It was then blotted with absorbent paper to dryness. A microscope was used to examine the staining procedure's results under oil immersion. Gram-negative bacteria stained pink/red at the end of the Gram Stain, while gram-positive bacteria stained purple (Claus, 1992) .

Tests Used to Identify Gram Positive Bacteria

Mannitol Salt Agar (MSA)

During this test, 111.025 gm of mannitol salt agar was suspended in 1000 ml of distilled water and then heated until it was dissolved. It was then autoclaved at 121 ºC for 15 min and then cooled to 45ºC before being poured into petri dishes. The MSA was selective for organisms that can survive in environments with heavy salt concentrations, such as *Staphylococcus* species. This was opposite to the *Streptococcus* species, where its growth was inhibited by the high levels of salt in the agar. Mannitol, a sugar, was the differentiating ingredient in MSA. Organisms that used mannitol as a source of food produced acidic fermentation by-products, which reduced the media's pH. The pH indicator, phenol red, turned yellow when the media became acidic. *Staphylococcus aureus* fermented mannitol whereas, *Staphylococcus epidermidis* could not ferment mannitol (Kateete et al., 2010).

Bile Esculin Agar (BEA)

A four-quadrant streak was made on to the BEA plate using tea sample bacterial isolates. It was then incubated anaerobically at 35 ºC and was observed after 24 h for esculinase positive colonies. They were held up to 72 h before being reported.

This was used in identification of members of the genus *Enterococcus* (*E. faecalis* and *E. faecium*). The product esculetin was generated when the test microorganism hydrolyzed esculin in the presence of bile. Esculetin combined with ferric citrate (in the medium) to generate a phenolic iron complex that darkened leading to the blackening of the entire slant. *Staphylococcus aureus* had good growth in this media and caused light blackening of the media (Bullock et al., 2013). This test was then followed by the catalase test.

Catalase Test

A microscope slide was placed in a petri-dish. Using a sterile inoculating loop, a small amount of organism was picked from a well-isolated 24 h colony from NA medium and placed on to the microscope slide. Care was taken not to pick any agar because carryover of red blood cells into the test could result in a false positive reaction. One (1) drop of 3% hydrogen peroxide was placed onto the organism on the microscope slide using a dropper and not mixed. To improve readability, the petri-dish was instantly covered to restrict aerosols and afterwards examined for immediate bubble formation against a dark background. This test was used to identify organisms that produce the enzyme, catalase. Hydrogen peroxide (H_2O_2) was detoxified by the enzyme, breaking it down into water and oxygen gas. A positive test was a bubbling reaction caused by the release of oxygen (O_2) from the H_2O_2 in the presence of catalase. In the absence of any bubbling reaction it was considered a negative test (Bullock et al., 2013). This test helped to distinguish between *staphylococci* and s*treptococci* because they both cause blackening of bile esculin agar medium. This was due to the possession of the catalase enzyme by *staphylococci* (Bullock et al., 2013).

Sulfur Indole Motility Media (SIM)

A well-isolated colony from a pure culture grown in a NA plate was picked using a straight inoculating needle. It was then inoculated by stabbing the middle of the sulfur indole motility medium tube at 2/3 the depth of the medium. The tubes were then incubated for 24 h and then observed. This tested the ability of an organism to reduce sulfur, produce indole and swim through. The agar SIM was used to differentiate members of

Enterobacteriaceae. Sulfur Indole Motility medium tubes were inoculated with a single stab to the bottom of the tube. If an organism was motile then the growth radiated from the stab mark and made the entire tube appear turbid. *Staphylococci spp* was not be motile (Pollitt et al., 2016) while on the other hand, *E. coli* and *Salmonella spp* were motile (Partridge and Harshey, 2013).

Tests Used to Identify Gram Negative Bacteria

MacConkey Agar

The plates were inoculated by directly streaking the sample from the NA enrichment media on the MacConkey agar surface. The Macconkey agar was prepared by weighing 49.53g of the agar and suspending it in 1000 ml of distilled water and then heating it to boil with agitation to completely dissolve. The media was then dispended in petri dishes. The inoculated plates were then incubated under anaerobic conditions for 24 h at 37ºC. Fermentation of lactose in the agar medium resulted in an acidic pH and caused the pH indicator, neutral red, to turn a bright pinky-red colour and this was used to differentiate between the *Enterobacteriaceae.* Pink colonies on MacConkey media which were indole positive were considered positive for *E. coli* (Tanih et al., 2015).

Simmon's Citrate Agar

About 24.2g of Simmon's Citrate Agar was suspended in 1000 ml of distilled water and heated to boil with agitation to dissolve completely. The media was then dispended into test tubes and sterilized by autoclaving at 121ºC for 15 min. The medium was then allowed to cool in a slanted position for use as slants.

It was utilized to distinguish between *Enterobacteriaceae* members. The enzyme citrase hydrolyzed citrate into oxaoloacetic acid and acetic acid in organisms that used citrate as a carbon source. After that, the oxaloacetic acid was hydrolyzed to produce pyruvic acid and carbon dioxide (CO_2). When CO_2 was generated, it interacted with the medium's components to form an alkaline compound. The pH indicator (bromthymol blue) changed colour from green to blue when the pH became alkaline. *Escherichia coli* were citrate negative (Vaughn et al., 1950).

3.6.2 Fungal Assay

3.6.2.1 Determination of Fungal (Yeast and Moulds) Load

This was done by weighing 50mg of the tea samples and infusing with 1ml of cold distilled water. One-hundred (100) µL of the tea samples was then inoculated in Potato Dextrose agar (PDA) at 25ºC for 48 h for fungal growth. Sterilized distilled water was used as a negative control. The colonies were then counted and counts expressed as colony forming units per gram (CFU g^{-1}).

3.6.3 Aflatoxin Analysis Using High Performance Liquid Chromatography (HPLC)

Chloroform was used to extract the cultures and the extract was concentrated in a vacuum. The dry material was then placed in 1ml vials with a tiny portion of chloroform. The solution was then evaporated to dryness under a stream of nitrogen. The crude extract was cleaned up by a silica gel column. About 1ml of 85% acetonitrile was added, mixed for 30

s and centrifuged at 1000 rpm for 15 min. HPLC was used to analyze the resulting supernatant (Viswanath et al., 2012).

Standard solutions of aflatoxin (0.5, 1, 5, 25, and 50 µg/L) were prepared in acetonitrile and subjected to derivatization. This involved being placed in an electric oscillator for 30 min and then filtering through a qualitative filter paper; 4 ml was then removed for purification. A TCM160 column was used for purification. Approximately 2 ml of the purified solution was transferred to a derivatization bottle, heated in a water bath at 60ºC and dried under a stream of nitrogen gas. Two-hundred (200) µL hexane (200 µl) and 100 µL trifluoroacetic acid (TFA) was added, mixed for 30 sec, and heated at 40ºC for 15 min. The mixture was then dried under a stream of nitrogen gas. The crude extract was cleaned up by silica gel column. About 1 ml of 85% acetonitrile was added, mixed for 30 sec and centrifuged at 1000 rpm for 15 min. The resulting supernatant was subjected to HPLC just like the extract from cultured tea samples (Wengui et al., 2015).

The aflatoxin concentrations in the tea samples were quantified by comparing the peak areas of the samples with those of aflatoxin standards.

The column type, temperature, emission wavelength, and flow rate of the fluorescence detector used in the HPLC system was optimized (Wengui et al., 2015).

3.7 Brewing of Made Tea

Tea samples of 0.5 g each were weighed and brewed with 10 ml autoclaved sterilized tap water in a beaker at 90 °C for 2 min. The brewed tea was decanted into another beaker after 2 min. The tea was left to cool down to room temperature (rtp) before inoculation on NA and PDA media for bacterial and fungal determination respectively (Peterson et al., 2004).

3.8 Moisture Determination

Approximately 2 g of made tea was weighed and dried in an oven for 24 h at 105 °C, to a constant weight. The sample was cooled in a desiccator and re-weighed to determine the final weight after drying. The moisture content was expressed as percentage of the dry weight. (You et al., 2018).

$$\% \text{ Moisture} = \frac{\text{Weight of sample before drying} - \text{ Weight of sample after drying}}{\text{Weight of sample before drying}} \times 100$$

3.8 Data Analysis and Presentation

Results of the parameters (colony forming units) determined were expressed as a mean of the triplicate determinations and were subjected to analysis of variance (ANOVA) using SAS version 9.1 (SAS. 2002) statistical software packages. The means, coefficient of variation and any difference between the samples was determined using ANOVA. The least significant differences Test (LSD) was used to separate the means. The probability limit was set at $p \leq 0.05$ significant level. The data and results were presented in for of tables, figures, graphs and pie charts.

3.9 Ethical Considerations

Ethical considerations in the characterization of microbes in processed Kenyan black CTC and green orthodox teas from selected tea factories encompassed the following aspects:

Informed Consent which involved obtaining voluntary, informed consent from participating factories, which ensured that they understood the purpose, procedures, risks, and benefits of the study. Protection of the confidentiality of participants' personal

information including participating factories and data collected during the study was also adhered to. Respect for participants and individual factory entities was ensured including participants' rights, autonomy, and privacy throughout the research process.

Scientific integrity as the research study was being conducted was adhered to as per accepted methodologies and standards. Stakeholder engagement was done and their perspectives were incorporated ensuring the study's relevance and benefit.

Ethics approval was obtained from National Commission for Science, Technology and Innovation (NACOSTI) before initiating the study and conduct ongoing ethical review and monitoring.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

This chapter discusses and presents the findings of the research study carried out on the characterization of microorganisms and toxins in made black cut tear and curl (CTC) and green teas along the tea value chain from selected Kenyan tea factories. It provides a comprehensive analysis of the results obtained from the research methods used in the various microbial analysis. Research findings include microbial profile of raw material, in process and finished products of Kenyan black CTC and green teas from selected factories. The quality of processed Kenyan black CTC teas from selected KTDA tea factories with respect to bacterial and fungal contamination of conformance to microbial quality standards in is also well illustrated. The findings of the profiling for aflatoxin in made teas that were contaminated with fungi is also be covered.

4.2 Microbial Quality of Raw Material, in Process and Finished Products of Kenyan Black CTC and Green Teas from Selected Tea Factories

At the point of reception of the tea leaves from the farm, the level of microbial contamination was highest as shown in Table 4.1. This is because tea leaves are collected from the farm and transported directly to the factory with no prior cleaning or washing. The tea leaves are usually contaminated with microbes from the soil and the ones introduced to it during harvesting by pluckers. Microbial contamination may also come from the bags used to carry leaf at harvesting in the field, handling and inspection at leaf

collection centers, loading into bags or creates at leaf collection centers and during transportation from transport vessels to the factories.

Some processing steps may help in reducing the microbial population in tea for instance the introduction of heat through steaming for the deactivation of polyphenol oxidase and polyphenol peroxidase enzymes in green tea processing. The steaming process is intended to stop oxidation but it additionally helps in eliminating some of the bacteria present in the tea leaves. The drying process, at 120℃ is sufficient to eliminate all the disease causing and most of the spoilage microorganisms present in the tea. Subsequent handling and packaging operations after drying may reintroduce microbes into the finished product. In the current study no bacteria was reintroduced into the tea during sorting, however this was not the case at the final stage of packaging, where of microbes were detected in Table 4.1.

Table 4.1:

Total bacterial count of microbes (CFU/g) found in green tea processing value chain of samples sourced from a multinational company.

Section	Leaf condition and status/ tea grade	Bacterial count (CFU)/g
Leaf reception	Fresh whole leaf	$>1.0x10^5$
Enzyme inactivation section	Steam-fixed leaf	$>1.0x10^5$
Cooling and Withering section	Cooled steamed leaf	$>1.0x10^5$
Maceration (CTC)	Cut Tear and Curl (CTC) dhool	$1.9x\ 10^3$
Drier Mouth	Dried tea of mixed grade from dryer	ND
Sorting section	Dried tea of mixed grade in bins	ND
Packaging	Dried tea of mixed in packaging material	$2.0x\ 10^2$

ND: No microbes detected

During the reception of fresh tea leaves from the farm, the level of microbial contamination was highest as seen in the Table 4.2 below. This is because the tea leaves were collected from the farm and transported directly to the factory without cleaning or washing. Contamination of the tea leaves occurred in the farm due to introduction of soil microbes and also during plucking. Microbial contamination could also have occurred during transportation since the materials used for packaging the tea leaves for example sacks and crates were not sterile.

Table 4.2:

Total bacterial count of microbes (CFU/g) found in the black tea processing value chain of samples sourced from a multinational company.

Section	Leaf condition, status/ Tea Grade	Bacterial Count(CFU/g)
Transportation (from lorry)	Fresh Whole leaf	$>1.0x10^5$
Leaf reception -Sorting	Fresh Whole leaf	$>1.0x10^5$
Withering troughs	Withered Whole leaf	$>1.0x10^5$
Maceration CTC1 Rollers	Macerated dhool	$>1.0x10^5$
Maceration CTC 2 Rollers	Macerated dhool	$>1.0x10^5$
Maceration CTC 3 Rollers	Macerated dhool	$>1.0x10^5$
Continuous Fermentation Unit 1 (CFU 1)	Macerated wet leaf	$>1.0x10^5$
Continuous Fermentation Unit 1 (CFU 2)	Macerated wet leaf	$>1.0x10^5$
Drier Mouth (DM)	Dried leaf ,Mixed grade	ND
Sorting section-Vibroscreen	BP1 grade sorted tea	ND
Sorting section-Vibroscreen	PD grade sorted tea	$6.2x10^2$
Sorting section-Vibroscreen	PF1 grade sorted	ND
Storage bin	BP1 grade-Bin stored tea	ND
Storage bin	PF1grade – Bin stored tea	ND
Storage bags	Mixed secondary grade teas	ND

ND: No microbes detected

The bacterial count during the processes of withering, maceration and fermentation did not exhibit much variation. The drying process, at 120℃ was sufficient to eliminate all the bacteria that were present in the processed tea leaves. During the sorting step, bacteria was reintroduced to grade PD of the tea, whereas BP1 andPF1 grades were not contaminated.

At the point of storage awaiting packaging the BP1, PF1 and secondary grades of teas were not contaminated. This was mainly attributed to the high level of hygiene that was kept at the multinational company tea processing factory.

At reception, the fresh tea leaves from the farm, have the highest level of microbial contamination as seen in the Table 4.3 below. This is because tea leaves being collected in the farm are not cleaned or washed. Tea leaves microbial contamination occurred in the farm due to introduction of soil microbes, during plucking or transportation since the materials used for packing the tea leaves for example sacks and crates were not sterile.

During the processes of withering, maceration and fermentation the level of bacterial contamination remained relatively constant. The drying process, at 120℃ was sufficient to eliminate all the bacteria that were present in the processed tea leaves. During the sorting bacteria was reintroduced to BP1, PD and PF1 grades of the tea. At the point of storage awaiting packaging the BP1, PD, PF1 and mixed secondary grades of teas were contaminated. This was mainly attributed to the laxity in keeping high level of hygiene at the private factory compared to that which was kept at the multinational company tea processing factory observed in Table 4.2.

The level of contamination at the point of sorting and storage was higher in the private tea factory compared to the multinational company tea factory owing to the stringent food safety measures and hygiene protocols adhered to at the multinational company. These findings provide valuable insights into the changes in the microbial profile of tea along the processing line to the finished product and their conformance to Kenyan standards and

provides information on the critical measures to be taken to ensure food safety of made teas at production and storage.

Table 4.3:

Total bacterial count of microbes (CFU/g) found in black tea processing value chain of samples sourced from a private factory

Section	Leaf Condition/ Tea Grade	Microbial Count CFU/g (Mean)
Leaf reception	Fresh Whole leaf	$>1.0x10^5$
Withering troughs	Withered Whole leaf	$>1.0x10^5$
Maceration CTC 1 Section	Withered macerated dhool	$>1.0x10^5$
Maceration CTC 2 Section	Withered macerated dhool	$>1.0x10^5$
Maceration CTC 3 Section	Withered macerated dhool	$>1.0x10^5$
Continuous Fermentation Unit 1 (CFU 1)	Fermented dhool	$>1.0x10^5$
Continuous Fermentation Unit 2	Fermented dhool	
Drier mouth	Mixed grade	ND
Sorting section-Vibroscreen	BP1 grade sorted tea	$6.9x10^3$
Sorting section-Vibroscreen	PD grade sorted tea	$1.5x10^3$
Sorting section-Vibroscreen	PF1 grade sorted tea	$2.8x10^4$
Storage Bin	BP1grade teas stored in bins	$2.3x10^4$
Storage Bin	PD grade tea stored in bins	$9.3x10^2$
Storage Bins	PF1grade tea stored in bins	$>1.0x10^5$
Storage Bags	Mixed secondary stored in bags	$3.5x10^4$

ND: No microbes detected

Escherichia coli and *Staphylococci spp*. were isolated from the samples. This is in agreement with a previous study whereby these bacteria had been detected in processed tea samples (Tan et al., 2016). *Salmonella spp*. was found to be 0 CFU/g in the made tea samples collected from both the private and multinational company tea factories. This shows that some teas did not meet the microbial quality standard requirements and there is need to improve on handling the teas to avoid contamination in the factories identified

since there should be no *E. coli* and *Staphylococci spp*. This can be clearly seen in tables 4.4, 4.5 and 4.6 which constitute of the findings and observations made on samples from a private factory and a multinational company. *E. coli* was the most abundant microbe found as a contaminant in both the green and black made teas.

Table 4.4:

Biochemical characterization of microbes from the black CTC processing line of samples sourced from a private factory.

Section	Leaf Condition/ Tea Grade	Selective Media				Biochemical Tests		Test bacteria	
		BEA	XLD	MCA	MSA	SIM	SCA	*E. coli*	*S. aureus*
Sorting section	BP1 tea in collection buckets	no growth	large, flat, yellow colony	pink colony	yellow colony with yellow zone	indole +ve motile, , no blackening	+ve	+ve	-ve
	PD tea in collection buckets	good growth with blackening of media	large, flat, yellow colony	pink colony	yellow colony with yellow zone	indole +ve motile, , no blackening	+ve	+ve	+ve
	PF1 tea in in collection buckets	good growth with blackening of media	large, flat, yellow colony	no growth	yellow colony with yellow zone	Indole +ve motile, , no blackening	+ve	+ve	+ve
Storage Bins	BP1 tea in storage bin tea	no growth	large, flat, yellow colony	pink colony	yellow colony with yellow zone	Indole +ve motile, , no blackening	+ve	+ve	-ve
	PD tea in storage bin	no growth	large, flat, yellow colony	no growth	no growth	indole -ve motile, , blackening	-ve	+ve	-ve
	Mixed secondary tea tea in storage buckets	no growth	no growth/red colony	no growth	no growth	indole –ve motile, , blackening	-ve	-ve	-ve

Selective media: BEA-Bile Esculin Agar,MCA-MacConkey Agar, MSA_Mannitol Salt Agar, XLD- Xylose Lysine Deoxycholate Biochemical tests:-SCA-Simmon Citrate Agar SIM-Sulphur Indole Motility media.+ve-positive ,-ve-negative

Table 4.5:

Biochemical characterization of microbes from the green tea processing line of teas samples sourced from a multinational tea company factory.

Section	Leaf condition/ tea grade	Morphological features				
		Gram staining	Shape	Colour	Margin	Elevation/consistency
Leaf Reception	Whole leaf	No growth	N/A	N/A	N/A	N/A
Enzyme Inactivation	Whole leaf	No growth	N/A	N/A	N/A	N/A
Maceration Cut Tear and Curl Machine (CTC 1)	Macerated leaf	G +ve	Rods	cream	regular	Flat (good growth & misty)
Maceration Cut Tear and Curl Machine (CTC 2)	Macerated leaf	No growth	N/A	N/A	N/A	N/A
Maceration Cut Tear and Curl Machine (CTC 3)	Macerated leaf	No growth	N/A	N/A	N/A	N/A
Dryer Mouth	Processed leaf	No growth	N/A	N/A	N/A	N/A
Packaging	Packaged leaf	G -ve	Rods	cream	regular	Raised (mucoid and viscous)

N/A-Not Applicable

Table 4.6:

Biochemical characterization of microbes from the black tea processing line of tea samples sourced from a multinational tea company factory.

Section	Leaf condition/ tea grade	Gram staining	Morphological features		
			Colour	Margin	Elevation/consistency
Leaf Reception	Fresh leaf	No growth	No growth	No growth	No growth
Withering	Withered leaf				
Maceration Cut Tear and Curl Machine (CTC 1)	Dhool	No growth	No growth	No growth	No growth
Maceration Cut Tear and Curl Machine (CTC 2)	dhool	No growth	No growth	No growth	No growth
Maceration Cut Tear and Curl Machine (CTC 3)	dhool	No growth	No growth	No growth	No growth
Continuous Fermenting Unit(CFU Section 1)	Fermented dhool	No growth	No growth	No growth	No growth
Continuous Fermenting Unit(CFU Section 2)	Fermented dhool	No growth	No growth	No growth	No growth
Dryer mouth	Mixed grades	No growth	No growth	No growth	No growth
Sorting section	BP1 Sorting	G-ve rods	cream	regular	Raised (mucoid and viscous)
	PD Sorting	G-ve rods	cream	regular	Raised (mucoid and viscous)
	PF1 Sorting	G-ve rods	cream	regular	Raised (mucoid and viscous)

Table 4.7:

Microbial and biochemical characterization of microbes of green tea samples sourced from a multinational tea company factory

Section	Leaf condition/ tea grade	Selective media				Biochemical tests		Test bacteria	
		BEA	XLD	MCA	MSA	SIM	SCA	*E.coli*	*S. aureus*
Leaf Reception	Fresh Leaf	No growth	No growth	No growth	No growth	No growth	No growth	-ve	-ve
Enzyme inactivation	Enzyme inactivated leaf	No growth	No growth	No growth	No growth	No growth	No growth	-ve	-ve
Withering Troughs	Withered leaf	No growth	No growth	No growth	No growth	No growth	No growth	-ve	-ve
Maceration CTC 1	Macerated leaf	No growth	No growth	No growth	yellow colony with yellow zone	motile, indole negative, blackening	Negative	-ve	+ve
Maceration CTC 2	Macerated leaf	No growth	No growth	No growth	No growth	No growth	No growth	-ve	-ve
Maceration CTC 3	Macerated leaf	No growth	No growth	No growth	No growth	No growth	No growth	-ve	-ve
Drier Mouth	Processed leaf	No growth	No growth	No growth	No growth	No growth	No growth	-ve	-ve
Packaging	Packaged leaf	no growth	large, flat, yellow colony	pink colony	no growth		Negative	+ve	-ve

Selective media:-BEA-Bile Esculin Agar,MCA-MacConkey Agar,MSA_Mannitol Salt Agar,XLD- Xylose Lysine Deoxycholate .Biochemical tests:-SCA-Simmon Citrate Agar SIM-Sulphur Indole Motility media.

Table 4.8:

Microbial and biochemical characterization of microbes of black tea samples sourced from a multinational tea company factory

Section	Leaf condition/ tea grade	Selective media				Biochemical tests		Test bacteria	
		BEA	XLD	MCA	MSA	SIM	SCA	*E coli*	*S. aureus*
Leaf Reception	Fresh Leaf	No growth	No growth	No growth	No growth	No growth	No growth	-ve	-ve
Withering Troughs	Withered Leaf	No growth	No growth	No growth	No growth	No growth	No growth	-ve	-ve
Maceration	Macerated dhool	No growth	No growth	No growth	No growth	No growth	No growth	-ve	-ve
CTC 1,2 and 3									
Dryer Mouth		No growth	No growth	No growth	No growth	No growth	No growth	-ve	-ve
Sorting section	BP1 Sorting	no growth	large, flat, yellow colony	pink colony	yellow colony with yellow zone	motile, indole negative, blackening	Positive	+ve	+ve
	PD Sorting	no growth	large, flat, yellow colony	pink colony	yellow colony with yellow zone	motile, indole negative, blackening	Negative	+ve	+ve
	PF1 Sorting	good growth, no blackening of media	red colony	clear colony	no growth	motile, indole negative, blackening	Negative	-ve	-ve
Packaging	Packaged leaf	no growth	large, flat, yellow colony	pink colony	no growth		Negative	-ve	-ve

Selective media:-BEA-Bile Esculin Agar,MCA-MacConkey Agar,MSA_Mannitol Salt Agar,XLD- Xylose Lysine Deoxycholate .Biochemical tests:-SCA-Simmon Citrate Agar SIM-Sulphur Indole Motility media, +ve-positive,-ve-negative

4.2.1 Growth of Bacterial Colonies on Selective Media

In Xylose Lysine Deoxycholate (XLD) media, *Salmonella spp.* form red colonies with black centres whereas *E. coli* form large, flat, yellow colonies. From this research study only the growth of isolated *E.coli* was observed as shown in Plate 4.1 below but no *Salmonella spp*. was observed when the bacterial isolates were inoculated in the XLD selective media. This showed that the made black CTC samples were mostly contaminated with *E.coli* but *Salmonella spp*. was not part of the microbial contaminants in the collected samples.

Plate 4.1: Plate showing the growth characteristics of *E. coli* on Xylose Lysine Deoxycholate (XLD) media.

Staphylococcus aureus had good growth in Bile Esculin Agar (BEA) media and caused light blackening of the media (Bullock et al., 2013). *E. coli* had good growth in BEA but with no blackening of media. Both *Staphylococcus aureus* and *E.coli* were found in some of the black made CTC samples in this research study as shown on the Plates 4.2 and 4.3 below. This showed that some of the tea samples could pose a health risk especially if consumed without proper boiling. In this context proper boiling of the made black CTC teas will mean that the consumer ensures that the water used for infusion of the teas has attained temperatures of about 100 ℃ so that the pathogenic microbes can be eliminated.

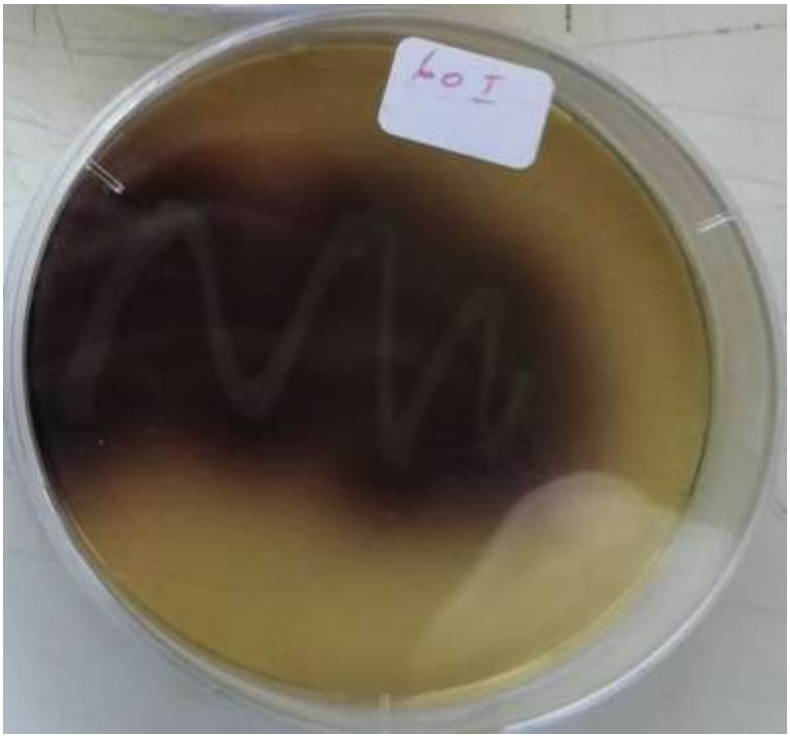

Plate 4.2: Plate showing the growth characteristics of *Staphylococcus aureus* on Bile Esculin Agar (BEA) media.

Plate 4.3: Plate showing the growth characteristics of *E.coli* on Bile Esculin Agar (BEA) media.

From Plate 4.4 below, *Staphylococcus aureus* used mannitol as a source of food and produced acidic fermentation by-products, which reduced the mannitol salt media's pH. The pH indicator, phenol red, turned yellow when the media became acidic. *Staphylococcus aureus* fermented mannitol and grew as yellow colonies with yellow zones (Kateete et al., 2010). This further confirmed the presence of *Staphylococcus aureus* in the made black CTC samples that was earlier observed in the Bile Esculin Agar (BEA). Proper hygienic measures needed to be adhered to in the tea factories to ensure that no microbes are introduced to the made teas once they get out of the drier mouth. This is because from this study it was discovered that the teas obtained from the drying step of processing were

sterile and that microbial contamination was reintroduced during the sorting and packaging operations which involved human handling.

Plate 4.4: Plate showing the growth characteristics of S*taphylococcus aureus* on mannitol salt Agar (MSA) media.

Pink colonies on MacConkey agar media which were indole positive were considered positive for *E. coli* (Tanih et al., 2015). Fermentation of lactose in the agar medium by *E.coli* resulted in an acidic pH and caused the pH indicator, neutral red, to turn a bright pinky-red colour as observed in Plate 4.5 below. This was further confirmation that the collected Kenyan made black CTC samples were contaminated with *E. coli*, this being was

the most common microbe isolated during the research study. Although most strains of *E. coli* are harmless some strains could be harmful (Kaper et al., 2004). The study did not unravel the particular strains of the isolated *E. coli* due to limited resources hence recommended for further work.

Plate 4.5: Plate showing the growth characteristics of E.coli on MacConkey agar media.

4.3 Isolation and Characterization of Bacteria in Processed Kenyan Black CTC from Selected KTDA Tea Factories before and after Brewing as Per Manufacturer's Instructions.

Isolation and characterization of bacteria was done on processed Kenyan Black CTC tea from selected KTDA tea factories and tables 4.9a-d, 4.10a-c and 4.11a-c show that some teas did not meet the microbial quality standard requirements and there is need to improve on handling the teas to avoid contamination in the factories identified. A total of 50 and 39 made tea samples were found to be contaminated with *E. coli* and *S. aureus* respectively from the total 126 collected made tea samples.

Table 4.9a:

Total bacterial count in CFU/g of primary and secondary grades black CTC made teas from selected KTDA tea factories in region 1 in the East of Rift Valley.

Factory	Tea Grade	Total Plate Count CFU/g	Kenya Standard-KS65 Requirement CFU/g
Factory 1	BP1	0	0
	PD	$2.3x\ 10^3$	0
	PF1	0	0
	F1	$1.3x10^2$	0
	Dust	0	0
	D1	0	0
Factory 2	BP1	$1.3x10^2$	0
	PF1	0	0
	PD	0	0
	F1	$1.8x10^3$	0
	D	$4.4x10^3$	0
	DI	$1.3x10^2$	0
	FBD I	0	0
	FBD IV	0	0

Factories 1 and 2 represent two selected coded factories of Region 1 of KTDA in the East of Rift Valley from which samples were drawn. BP-Broken Pekoe, PD-Pekoe Dust, PF-Pekoe Fanning, F-fanning, D-dust, FBD-fluid bed drier, Mnf-manufacture.

Table 4.9b:

Total bacterial count in CFU/g of primary and secondary grades black CTC made teas from selected KTDA tea factories in region 2 in the East of Rift Valley.

Factory	Tea Grade	Total Plate Count CFU/g	Kenya Standard-KS65 Requirement CFU/g
Factory 1	BP1	0	0
	PD	0	0
	PF1	0	0
	F1	0	0
	D1	0	0
	DII(Drier Mouth)	0	0
Factory 2	BP1	0	0
	PD	0	0
	PF1	0	0
	F1	$2.3x10^2$	0
	D	0	0
	DI	0	0

Factories 1 and 2 represent two selected coded factories of Region 2 of KTDA in the East of Rift Valley from which samples were drawn. BP-Broken Pekoe, PD-Pekoe Dust, PF-Pekoe Fanning, F-fanning, D-dust, FBD-fluid bed drier, Mnf-manufacture.

During sampling from the tea factories, the type of sampling was simple random sampling .The samples were collected from various points including bins, screens and sorting. Bins are used to store processed tea leaves helping maintain organization and prevent contamination. Screens are used to separate processed tea leaves based on size and quality, ensuring only the best leaves are processed. Sorting systems automate the categorization

of leaves, enhancing efficiency and quality control in tea production and ultimately resulting in the different tea grades.

Table 4.9c:

Total bacterial count in CFU/g of primary and secondary grades black CTC made teas from selected KTDA tea factories in region 3 in the East of Rift Valley.

Factory	Tea Grade	Total Plate Count CFU/g	Kenya Standard-KS65 Requirement CFU/g
Factory 1	BP1	0	0
	BP1(Bins)	0	0
	PD	0	0
	PD(Bins)	0	0
	PF1	0	0
	PF1(Bins)	$1.33x10^2$	0
	Fannings	$2.7x10^2$	0
	D	4.7	0
	D1	0	0
	DII	0	0
Factory 2	BP1 (screens)	0	0
	BP1 (bins)	0	0
	PD(sorting)	0	0
	PD (bins)	0	0
	PF1	0	0
	PF1(bins)	0	0
	F1	$1.3x10^2$	0
	D(screen)	$2.1x10^3$	0
	D1 (bins)	0	0
	Drier I	0	0
	Drier II	0	0

Factories 1 and 2 represent two selected coded factories of Region 3 of KTDA in the East of Rift Valley from which samples were drawn. BP-Broken Pekoe, PD-Pekoe Dust, PF-Pekoe Fanning, F-fanning, D-dust, FBD-fluid bed drier, Mnf-manufacture.

Table 4.9d:

Total bacterial count in CFU/g of primary and secondary grades black CTC made teas from selected KTDA tea factories in region 4 in the East of Rift Valley.

Factory	Tea Grade	Total Plate Count CFU/g	Kenya Standard-KS65 Requirement CFU/g
Factory 1	BP1	0	0
	PD	0	0
	PF1	0	0
	F1	$6x10^1$	0
	D1	$6.4x10^3$	0
	DII	$1.0x10^3$	0
Factory 2	BP1(Mnf)	0	0
	PD	0	0
	PF1(Mnf)	0	0
	PF1(bins)	0	0
	F1(Mnf)	$7.3x10^2$	0
	F1(Sacks)	$4.3x10^4$	0
	D(Mnf)	$2.1x10^4$	0
	D(Sacks)	$2.2x10^3$	0
	D1(Mnf)	$2.7x10^2$	0
	FBD I	$3.3x10^2$	0
	FBD II	0	0

Factories 1 and 2 represent two selected coded factories of Region 4 of KTDA in the East of Rift Valley from which samples were drawn. BP-Broken Pekoe, PD-Pekoe Dust, PF- Pekoe Fanning, F-fanning, D-dust, FBD-fluid bed drier, Mnf-manufacture.

Table 4.10a:

Total bacterial count in CFU/g of primary and secondary grades black CTC made teas selected KTDA from region 5 tea factories in the West of Rift Valley.

Factory	Tea Grade	Total Plate Count CFU/g	KenyaStandard-KS65 Requirement CFU/g
Factory 1	BP1	0	0
	PD	0	0
	PF1	0	0
	F1	$1.6x10^4$	0
	DI	$4.7x10^2$	0
	D	0	0
	TMF	$7.7x10^3$	0
	DM1	$7.9x10^3$	0
	DMII	0	0
Factory 2	BP1 (Bins)	$3.3x10^2$	0
	PD (Bins)	0	0
	PF1 (Bins)	0	0
	D1 (Bins)	0	0
	BP1	0	0
	PD	$1.6x10^3$	0
	PF1	0	0
	F1	$9.2x10^3$	0
	D	$6.5x10^3$	0
	DI	$3.3x10^2$	0
	Drier Mouth 1	$3.3x10^2$	0
	Drier Mouth 2	0	0
	Drier Mouth 3	0	0

Region 5 represents true regions of the KTDA factories. Factories 1 and 2 represent two selected coded factories from in the KTDA region in the West of Rift Valley from which samples were drawn. BP-Broken Pekoe, PD-Pekoe Dust, PF- Pekoe Fanning, F-fanning, D-dust, DM-Drier Mouth, TMF-Total Mixed Fannings.

Table 4.10b:

Total bacterial count in CFU/g of primary and secondary grades black CTC made teas from regions 6 of selected KTDA tea factories in the West of Rift Valley.

Factory	Tea Grade	Total Plate Count CFU/g	KenyaStandard-KS65 Requirement CFU/g
Factory 1	BP1 (Bins)	$2.6x10^3$	0
	PD (Bins)	0	0
	PF1 (Bins)	0	0
	D (Bins)	0	0
	BP1	$2.0x10^2$	0
	PD	0	0
	PF1	$6.5x10^3$	0
	F1	0	0
	D	$1.8x10^3$	0
	DI	0	0
	TMF	$3.3x10^2$	0
	DM1	0	0
	DM2	0	0
	BP1 (Bins)	0	0
	PD (Bins)	0	0
	PF1 (Bins)	$5.3x10^3$	0
	DI (Bins)	$1.3x10^2$	0
Factory 2	BP1	$3.3x10^2$	0
	PD	$1.7x10^3$	0
	PF1	0	0
	F1	$5.3x10^2$	0
	D	$2.0x10^3$	0
	DI	$3.9x10^3$	0
	DM1	0	0
	DM2	0	0

Region 6 represents true regions of the KTDA factories. Factories 1 and 2 represent two selected coded factories from in the KTDA region in the West of Rift Valley from which samples were drawn. BP-Broken Pekoe, PD-Pekoe Dust, PF- Pekoe Fanning, F-fanning, D-dust, DM-Drier Mouth, TMF-Total Mixed Fannings.

Table 4.10c:

Total bacterial count in CFU/g of primary and secondary grades black CTC made teas from region 7 selected KTDA tea factories in the West of Rift Valley.

Factory	Tea Grade	Total Plate Count CFU/g	KenyaStandard-KS65 Requirement CFU/g
Factory 1	BP1	$1.9x10^3$	0
	PD	0	0
	PF1	0	0
	F1	$1.3x10^2$	0
	D	0	0
	DI	$1.4x10^3$	0
Factory 2	BP1	$6.7x10^1$	0
	PD	$6.7x10^1$	0
	PF1	$6.7x10^1$	0
	F	$6.7x10^1$	0
	D	$2.0x10^2$	0
	DI	0	0
	DM1	0	0
	DM2	0	0
	DM3	0	0

Region 7 represents true regions of the KTDA factories. Factories 1 and 2 represent two selected coded factories from in the KTDA region in the West of Rift Valley from which samples were drawn. BP-Broken Pekoe, PD-Pekoe Dust, PF- Pekoe Fanning, F-fanning, D-dust, DM-Drier Mouth, TMF-Total Mixed Fannings.

Table 4.11a:

Analysis of bacterial colony forming units per gram in made black CTC tea from selected Kenyan factories in the East of Rift Valley region.

Region	Factory/Grade	BP1	PD	PF1	F1	D	D1	Mean	LSD	CV
1	1	ND	ND	ND	6.04×10^{1}	ND	ND	1.55×10^{0}	2.215	96.15
	2	ND	ND	ND	ND	ND	ND	ND	6.00E-17	4.78E-15
2	1	ND	ND	ND	5.56×10^{0}	4.44×10^{3}	1.62×10^{3}	1.68×10^{1}	2.767	51.835
	2	ND	2.3×10^{3}	ND	9.72×10^{0}	2.30×10^{3}	ND	2.62×10^{1}	2.249	37.017
3	1	ND	ND	ND	6.84×10^{2}	2.09×10^{4}	6.04×10^{1}	4.18×10^{1}	2.31	33.571
	2	ND	ND	ND	4.14×10^{3}	1.52×10^{3}	ND	8.09×10^{0}	2.043	48.585
4	1	ND	ND	ND	6.66×10^{1}	3.43×10^{2}	ND	6.50×10^{0}	2.504	64.29
	2	4.14×10^{1}	ND	ND	1.68×10^{3}	3.95×10^{3}	4.14×10^{3}	5.86×10^{1}	3.007	40.257
	MEAN	0.94	2.84	ND	47.81	450.82	5.84			
	LSD	1.65	0.031	1.70E-16	3.472	1.139	3.387			
	CV	87.42	1.13	1.40	50.73	10.63	93.90			

Regions 1-4 represent true regions of the KTDA factories. Factories 1 and 2 represent two selected coded factories from in the KTDA region in the East of Rift Valley from which samples were drawn. BP-Broken Pekoe, PD-Pekoe Dust, PF- Pekoe Fanning, F-fanning, D-dust, DM-Drier Mouth, TMF-Total Mixed Fannings, ND-no microbes detected, LSD-Least Significant Difference, CV-Coefficient of Variance.

Table 4.11b:

Analysis of bacterial colony forming units per gram in made black CTC tea from selected Kenyan factories in the West of Rift Valley Region.

Region	Factory/Grade	BP1	PD	PF1	F1	D	D1	Mean	LSD	CV
5	1	1.93×10^3	ND	ND	4.14×10^1	ND	8.44×10^2	26.78	2.06	33.63
	2	ND	1.24×10^3	ND	9.10×10^3	6.12×10^3	6.67×10^1	161.45	2.55	27.49
6	1	ND	ND	ND	1.55×10^4	9.68×10^1	ND	15.05	2.55	49.45
	2	5.26×10^1	ND	3.98×10^3	ND	1.77×10^3	ND	36.14	2.33	35.11
7	1	7.31×10^0	7.31×10^0	7.31×10^0	7.31×10^0	5.25×10^1	ND	7.68	2.86	69.30
	2	3.17×10^2	1.64×10^3	ND	5.24×10^2	1.46×10^3	3.17×10^3	368.03	1.05	9.77
MEAN		43.10	21.11	7.17	195.85	233.69	31.75			
LSD		2.738	2.041	2.012	2.652	3.647	2.654			
CV		30.846	35.722	49.922	27.571	36.702	41.448			

Regions 5-7 represent true regions of the KTDA factories. Factories 1 and 2 represent two selected coded factories from in the KTDA region in the West of Rift Valley from which samples were drawn. BP-Broken Pekoe, PD-Pekoe Dust, PF- Pekoe Fanning, F-fanning, D-dust, DM-Drier Mouth, TMF-Total Mixed Fannings, ND-no microbes detected, LSD-Least Significant Difference, CV-Coefficient of Variance.

Table 4.11c:

Overall analysis of bacterial colony forming units per gram in made black CTC tea from selected Kenyan factories in both East and West of Rift Valley regions.

		BP1	PD	PF1	F1	D	D1
Overall MEAN		6.27c	7.45c	1.84d	87.94d	340.27a	12.66d
Overall LSD for Grades	0.5923						
Overall LSD for Factories	0.9048						
LSD for Reps	0.4188						
Overall CV	44.0567						

BP-Broken Pekoe, PD-Pekoe Dust, PF- Pekoe Fanning, F-fanning, D-dust, DM-Drier Mouth, TMF-Total Mixed Fannings, ND-no microbes detected, LSD-Least Significant Difference, CV-Coefficient of Variance.

The secondary grades had higher levels of contamination compared to the primary tea grades areshown in the pie chart (Figure 4.1). From the chart below primary tea grades Broken Pekoe (BP), Pekoe Fannings (PF), Pekoe Dust (PD) had the least levels of bacterial contamination whereas the secondary grades Dust (D) and Fannings (F) had higher levels of contamination.

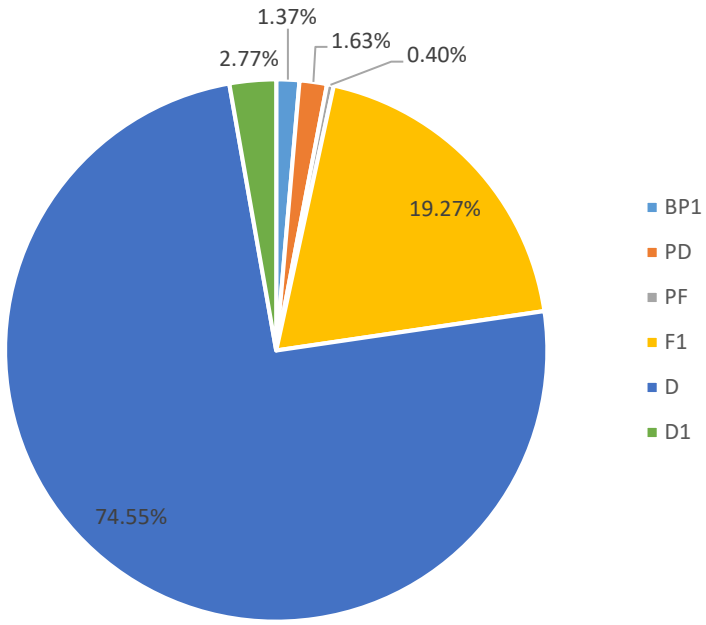

Figure 4.1: Level of bacterial contamination in the different grades of black CTC teas from selected Kenyan Tea factories.

BP-Broken Pekoe, PD-Pekoe Dust, PF- Pekoe Fanning, F-fanning, D-dust.

Dust exhibited the highest level of contamination, attributed to its very fine particulate size, which increases the surface area available for adsorbing contaminants. This contrasts with Fannings (F) tea grade, which, while also categorized as a secondary grade, has larger

particle sizes and consequently a reduced surface area for contamination (Ochanda and Ruto 2021). This is because equal amounts of samples were being weighed to check for microbial contamination regardless of particle size.

The Figure 4.2 shows the different grades of tea that are obtained during the sorting stage of tea processing. The different grades are obtained by sorting the mixed processed teas taken from the drier mouth and then subjecting them to shaking on top of meshes of different sizes arranged in descending pore sizes to separate the different tea grades (Ochanda and Ruto, 2022).

Figure 4.2: Tea Grading for Kenya tea industry

The primary grades include Broken Pekoe (BP), Pekoe Fannings (PF), Pekoe Dust (PD) and are usually obtained under first three meshes whereas the secondary grades are :-Dust (D) and Fannings (F) and are obtained in the subsequent final meshes below. The primary grades are mostly meant for export and local consumption while most of the secondary grades are used for other non-food products for example the Fannings (F) tea grade are used to make of ceiling boards. As a result the primary grades are handled with more hygiene compared to the secondary grades at the point of sorting and packaging operations. Therefore the secondary tea grades are more susceptible to contamination by handling operations even though all the tea grades are usually free from microbial contamination when they are being taken out from the drier mouth, where the temperatures were high.

The level of bacterial contamination is highest in Dust (D) tea grade from factory number 5 followed by Fannings (F) tea grade in factory number 11 as shown in Figure 4.3.The secondary grades, that is the dust and Fannings (F) tea grade are more visible in the chart as their levels are higher than those of the primary grades (BP, PF and PD) under the scale used.

Bacterial contamination was generally higher in the sampled made black CTC tea grades from the West of Rift Valley compared to the East of Rift Valley except for the Dust grade, as evidenced in the Figure 4.4. This could be attributed to policies of food safety and hygiene being adhered to more strictly in the East of Rift Valley compared to the West of Rift Valley .It should be noted that the made black CTC teas from the East of Rift Valley regions usually fetch way higher auction prices compared to the West of Rift Valley(

Chepkwony et al., 2023). This is mainly attributed to the tea quality. From the results, it is interesting to note that the East of Rift Valley made black CTC teas are generally less contaminated with microbes compared to their counterparts in the West of Rift Valley region.

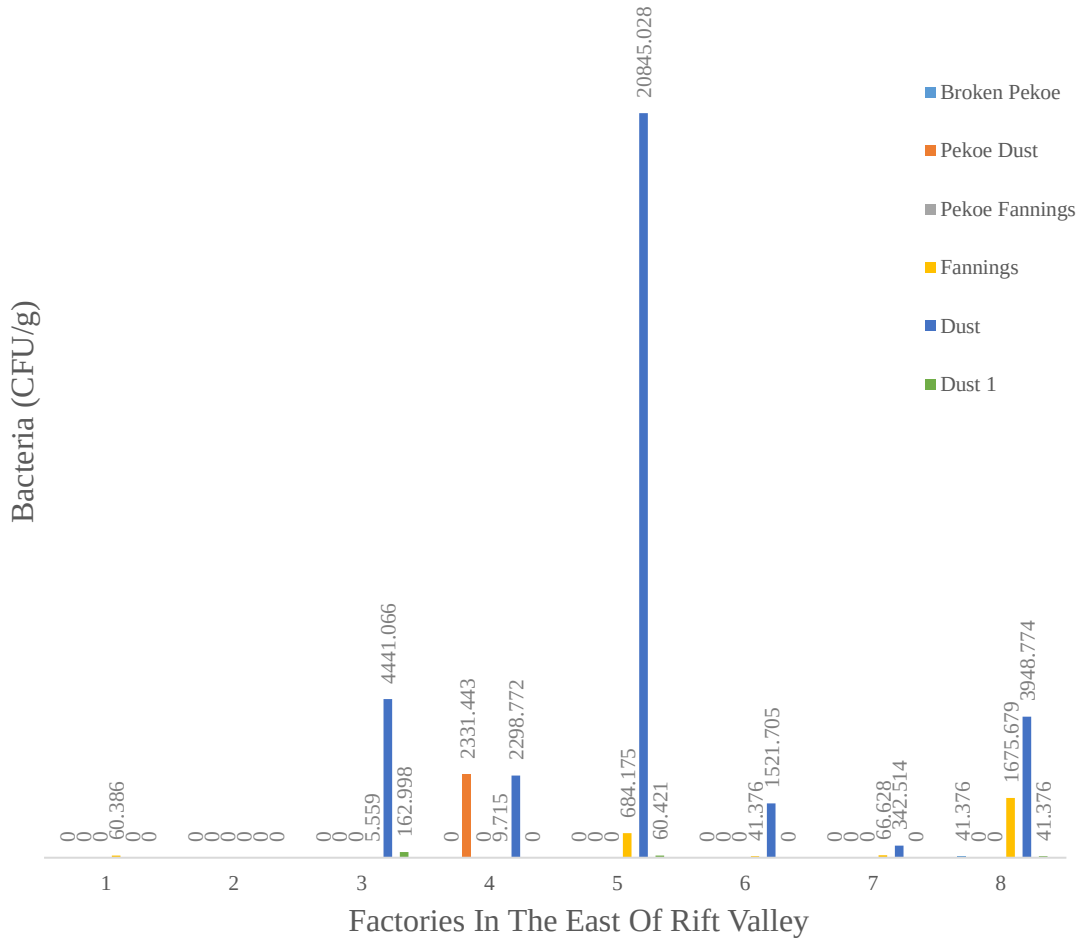

Figure 4.3a: Bar chart showing comparison of the level of bacterial contamination in CFU/g (Y-axis) against the different types of tea grades in 8 selected Kenyan tea factories (X-axis) from the East of Rift Valley. The numbers 1-8 code for real Kenyan tea factories from the East of Rift Valley regions from which the samples were collected.

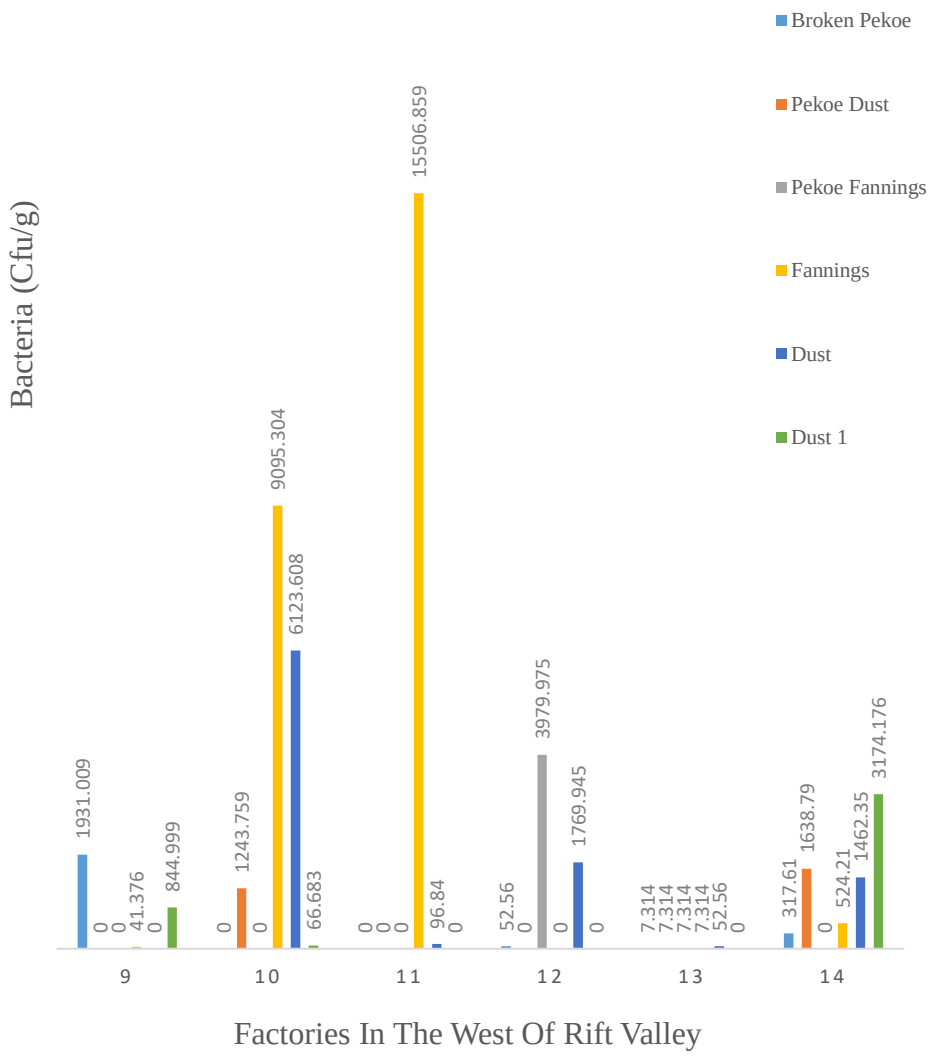

Figure 4.3b: Bar chart showing comparison of the level of bacterial contamination in CFU/g (Y-axis) against the different types of tea grades in 6 selected Kenyan tea factories (X-axis) from the West of Rift Valley. The numbers 9-14 code for real Kenyan tea factories from the West of Rift Valley regions from which the samples were collected.

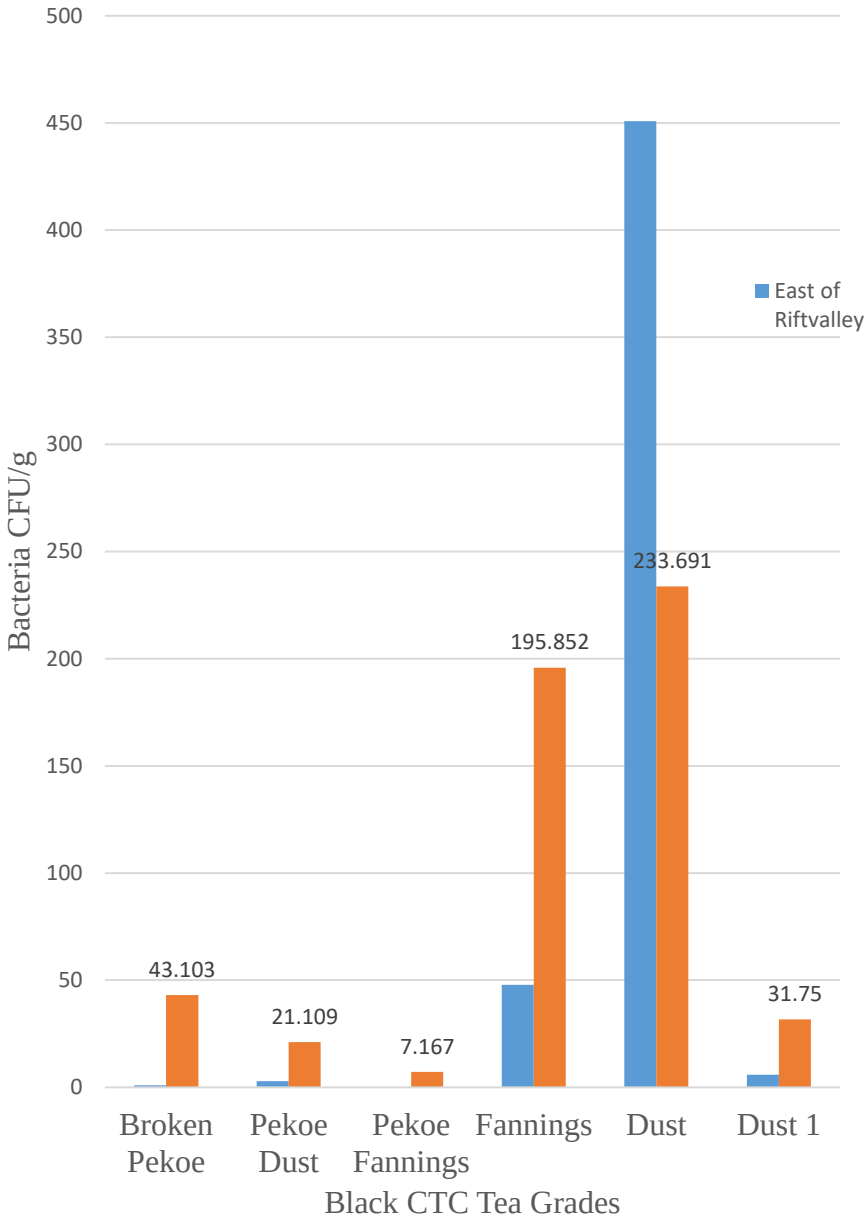

Figure 4.4: Bar chart comparing the level of bacterial contamination between the East And West of Rift Valley regions in the different made black CTC tea grades.

4.3.1 Isolation and Characterization of Bacteria in Processed Kenyan Made Black CTC from Selected KTDA Tea Factories after Brewing as Per Manufacturer's Instructions

A total of 9 black CTC made tea samples out of the 126 collected samples were found to contain bacteria even after brewing using boiling water at 100 ℃.This shows the existence of heat-resistant bacteria in the black made CTC tea samples and causes great concern as they may pose a health risk (Bintsis, 2017). Among them were some heat-resistant *E. coli* and heat-resistant *Staphylococcus aureus* .There was no heat-resistant *Salmonella spp* identified.

All the samples identified to be contaminated pathogenic bacteria were from the East of Rift Valley .They consisted of:-3 primary, 3 secondary and 2 mixed tea grades black made CTC samples.

In conclusion, high levels of hygiene should be upheld in the affected factories to avoid cross-contamination of the made teas as these are disease-causing bacteria (Gizaw, 2019).

4.4 Isolation and characterization of Yeast and Moulds in Processed Kenyan Made Black CTC Teas from Selected KTDA Tea Factories

The results of the isolation and characterization of yeast and moulds of the eight factories from which samples were collected in the east of Rift Valley, six factories had samples contaminated with yeast and moulds. Tables 4.10a-d clearly illustrates this. The remaining two factories' samples were completely free from yeast and moulds contamination. This

contamination was mostly in the secondary grades, that is Dust (D) and Fannings (F) except for factory two in region one where PF grade and factory 1 in region 3 where the PD grade is contaminated with yeast and moulds.PF and PD are primary grades. It was observed that the secondary grades are generally more contaminated with yeast and moulds compared to the primary grades. This difference was brought about by the difference in the way these grades are handled and packaged. The primary grades are handled aseptically and stored in new sterile aluminum-lined brown bags whereas the secondary grades are handled with less care and stored in recycled sacks.

Table 4.12a:

Presence of yeast and moulds in primary and secondary grades of black CTC made teas from region 1 of KTDA tea factories in the East of Rift Valley.

Factory	Identity of the sample	Presence of yeast and moulds
Factory 1	BP1	ND
	PD	ND
	PF1	ND
	F1	Present
	D	Present
	DI	ND
Factory 2	BP1	ND
	PD	ND
	PF1	Present
	F1	ND
	D1	Present
	DII(drier mouth)	ND

Factories 1 and 2 represent two selected coded factories from the East of Rift Valley from which samples were drawn. BP-Broken Pekoe, PD-Pekoes Dust, PF- Pekoe Fanning, F-Fanning, D-dust, FBD-fluid bed drier, Mnf-manufacture, DM-drier mouth.

Table 4.12b:

Presence of yeast and moulds in primary and secondary grades of black CTC made teas from region 2 of KTDA tea factories in the East of Rift Valley.

Factory	Identity of the sample	Presence of yeast and moulds
Factory1	BP1	ND
	PD	ND
	PF1	ND
	F1	ND
	D1	ND
	DII	ND
Factory 2	BP1	ND
	PD	ND
	PF1	ND
	F1	Present
	Dust	Present
	D1	ND

Factories 1 and 2 represent two selected coded factories from the East of Rift Valley from which samples were drawn. BP-Broken Pekoe, PD-Pekoes Dust, PF- Pekoe Fanning, F-Fanning, D-dust, FBD-fluid bed drier, Mnf-manufacture, DM-drier mouth.

Table 4.12c:

Presence of yeast and moulds in primary and secondary grades of black CTC made teas from region 3 of KTDA tea factories in the East of Rift Valley.

Factory	Identity of the sample	Presence of yeast and moulds
Factory 1	BP1(Mnf)	ND
	PD	Present
	PF1(Mnf)	ND
	PF1(bins)	ND
	F1(mnf)	Present
	F1(sacks)	Present
	D(mnf)	Present
	D(sacks)	Present
	D1(mnf)	ND
	FBD I	ND
	FBD II	ND
Factory 2	BP1 (screens)	ND
	BP1 (bins)	ND
	PD(sorting)	ND
	PD (bins)	ND
	PF1	ND
	PF1(bins)	ND
	F1	ND
	D(screen)	ND
	D1 (bins)	ND
	Drier I	ND
	Drier II	ND

Factories 1 and 2 represent two selected coded factories from the East of Rift Valley from which samples were drawn. BP-Broken Pekoe, PD-Pekoes Dust, PF- Pekoe Fanning, F-Fanning, D-dust, FBD-fluid bed drier, Mnf-manufacture, DM-drier mouth.

Table 4.12d:

Presence of yeast and moulds in primary and secondary grades of black CTC made teas from region 4 of KTDA tea factories in the East of Rift Valley.

Factory	Identity of the sample	Presence of yeast and moulds
Factory 1	BP1	ND
	BP1(bins)	ND
	PD	ND
	PD(bins)	ND
	PF1	ND
	PF1(bins)	ND
	Fannings (F)	Present
	D	Present
	D1	ND
	DII	Present
Factory 2	BP1	ND
	PF1	ND
	PD	ND
	F1	Present
	D	ND
	DI	ND
	FBD I	ND
	FBD IV	ND

Factories 1 and 2 represent two selected coded factories from the East of Rift Valley from which samples were drawn. BP-Broken Pekoe, PD-Pekoes Dust, PF- Pekoe Fanning, F-Fanning, D-dust, FBD-fluid bed drier, Mnf-manufacture, DM-drier mouth.

Table 4.11a-c shows that three out of the six were contaminated with yeast and moulds, whereas the remaining three factories were free from yeast and moulds contamination. Both primary and secondary grades were contaminated. This could have been because of laxity in implementation of food safety measures and hygiene protocols implementation in

the factories as observed in Figure 4.9 as well for bacterial contamination. Strict adherence to food safety protocols eliminates microbial contamination as observed in the factories in region 6.

From Table 4.10a-d and 4.11a-c, a total of 23 out of the 126 made black CTC samples collected from the 7 regions in both the east and west of Rift Valley were contaminated with yeast and moulds but the levels of microbial contamination were less than 10^3 CFU/g .They were thus within the acceptable limits in KS 65 of the Kenyan made black tea standards. Storage fungi have also been found in previous research studies and they were found to be significant contributors to tea degradation (Sedova et al., 2018).

Table 4.13a:

Presence of yeast and moulds in primary and secondary grades of black CTC made teas from selected KTDA tea factories in regions 5 in the West of Rift Valley.

Factory	Identity of the Sample	Presence of Yeast and Moulds
Factory 1	BP1	ND
	PD	ND
	PF1	ND
	F1	ND
	D	Present
	DI	ND
Factory 2	BP1	ND
	PD	ND
	PF1	ND
	F1	Present
	D	Present
	DI	ND
	Drier mouth 1	Present
	Drier mouth 2	ND
	Drier mouth 3	ND
	BP1 (Bins)	ND
	PD (Bins)	ND
	PF1 (Bins)	Present
	D (Bins)	Present

Factories 1 and 2 represent two selected coded factories from each of the KTDA region 5 in the West of Rift Valley from which samples were drawn. BP-Broken Pekoe, PD-Pekoes Dust, PF- Pekoe Fanning, F-fanning, D-dust, FBD-fluid bed drier, Mnf-manufacture.DM-drier mouth, DM-Drier Mouth, TMF-Total Mixed Fannings.

Table 4.13b:

Presence of yeast and moulds in primary and secondary grades of black CTC made teas from selected KTDA tea factories in regions 6 in the West of Rift Valley.

Factory	Identity of the Sample	Presence of Yeast and Moulds
Factory 1	BP1	ND
	PD	ND
	PF1	ND
	F1	ND
	DI	ND
	D	ND
	TMF	ND
	DM1	ND
	DMII	ND
	BP1 (Bins)	ND
	PD (Bins)	ND
	PF1 (Bins)	ND
	D1 (Bins)	ND
Factory 2	BP1	ND
	PD	ND
	PF1	ND
	F1	ND
	D	ND
	DI	ND
	TMF	ND
	DM1	ND
	DM2	ND
	BP1 (Bins)	ND
	PD (Bins)	ND
	PF1 (Bins)	ND
	DI (Bins)	ND

Factories 1 and 2 represent two selected coded factories from each of the KTDA region 6 in the West of Rift Valley from which samples were drawn. BP-Broken Pekoe, PD-Pekoes Dust, PF- Pekoe Fanning, F-fanning, D-dust, FBD-fluid bed drier, Mnf-manufacture.DM-drier mouth, DM-Drier Mouth, TMF-Total Mixed Fannings.

Table 4.13c:

Presence of yeast and moulds in primary and secondary grades of black CTC made teas from selected KTDA tea factories in regions 7in the West of Rift Valley.

Factory	Identity Of The Sample	Presence of Yeast and Moulds
Factory 1	BP1	ND
	PD	ND
	PF1	ND
	F	ND
	D	ND
	DI	ND
	DM1	ND
	DM2	ND
	DM3	ND
Factory 2	BP1	ND
	PD	present
	PF1	ND
	F1	ND
	D	ND
	DI	present
	DM1	ND
	DM2	ND

Factories 1 and 2 represent two selected coded factories from KTDA regions 7 in the West of Rift Valley from which samples were drawn. BP-Broken Pekoe, PD-Pekoes Dust, PF-Pekoe Fanning, F-fanning, D-dust, FBD-fluid bed drier, Mnf-manufacture.DM-drier mouth, DM-Drier Mouth, TMF-Total Mixed Fannings.

4.5 Determination of the Presence Aflatoxin in Made Black CTC Teas Contaminated with Yeast or Moulds from Selected KTDA Tea Factories.

4.5.1 Aflatoxin Analysis Using Thin Layer Chromatography (TLC)

The chromatogram in Figure 4.5 shows that made black tea sample 3 did not contain aflatoxin despite previously being contaminated with moulds. The maize sample and chicken feeds were used as in-house controls or standards to ascertain that the method of aflatoxin extraction was functional accurate and reliable. The extraction procedure was successful as evidenced by the presence of aflatoxin B1 in the chicken feeds and both B1 and B2 in the maize sample. This therefore shows that the tea sample was free from aflatoxin contamination.

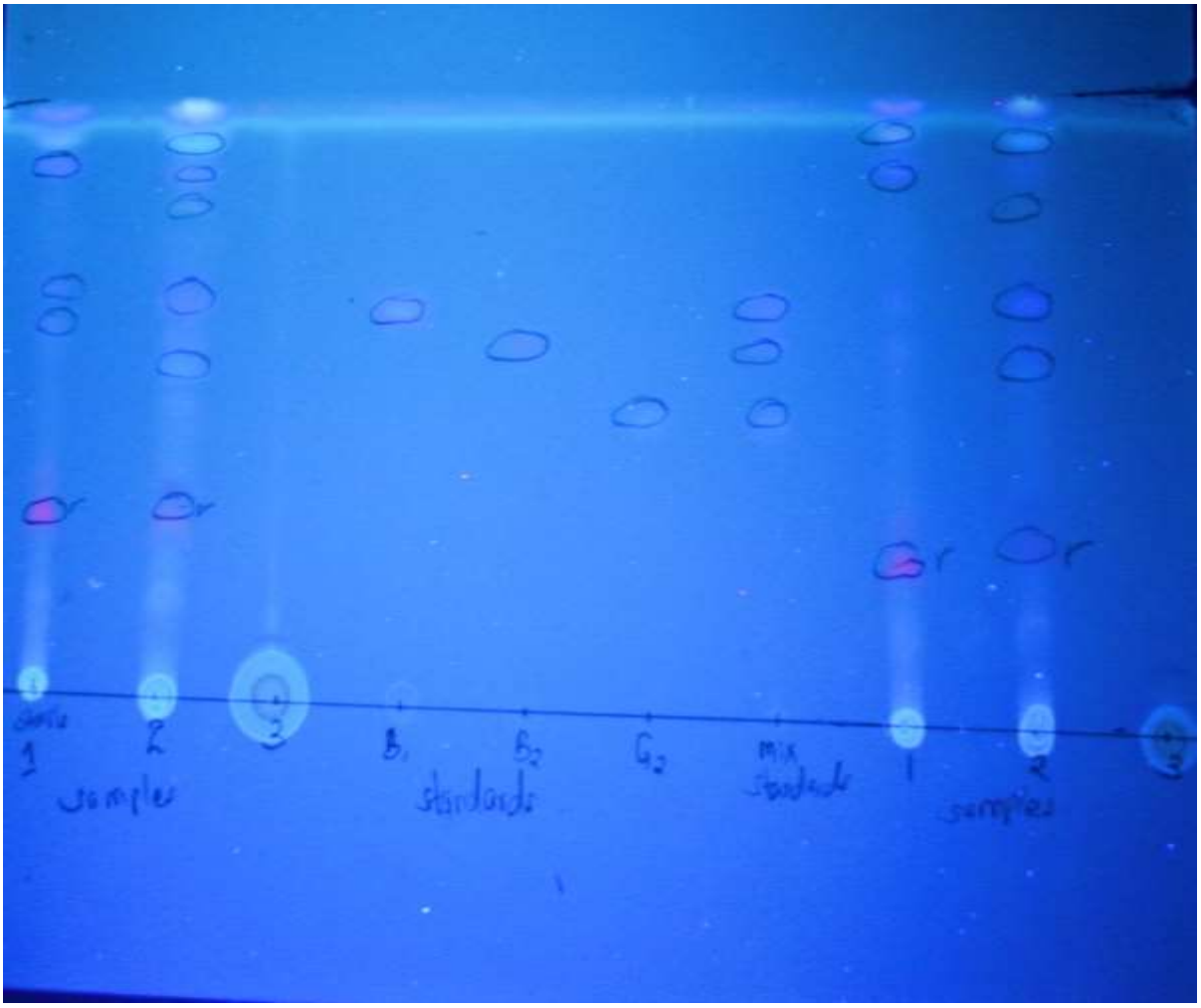

Figure 4.5: TLC plate with Chicken feeds, maize suspected to be contaminated with aflatoxin, aflatoxin standards and a made black CTC sample contaminated with yeast and moulds.

Sample 1-Chicken feeds; Sample 2- Maize; Sample 3-tea sample.

In Figure 4.6, the TLC plate was mounted with aflatoxin B1, B2, G2, chicken feed, a cocktail of the standards in equal ratios followed by 5 made black CTC samples. The made

black CTC tea samples in Figure 4.6 just like the one in Figure 4.5 were free from aflatoxin contamination. The chicken feed was used as an in-house positive control.

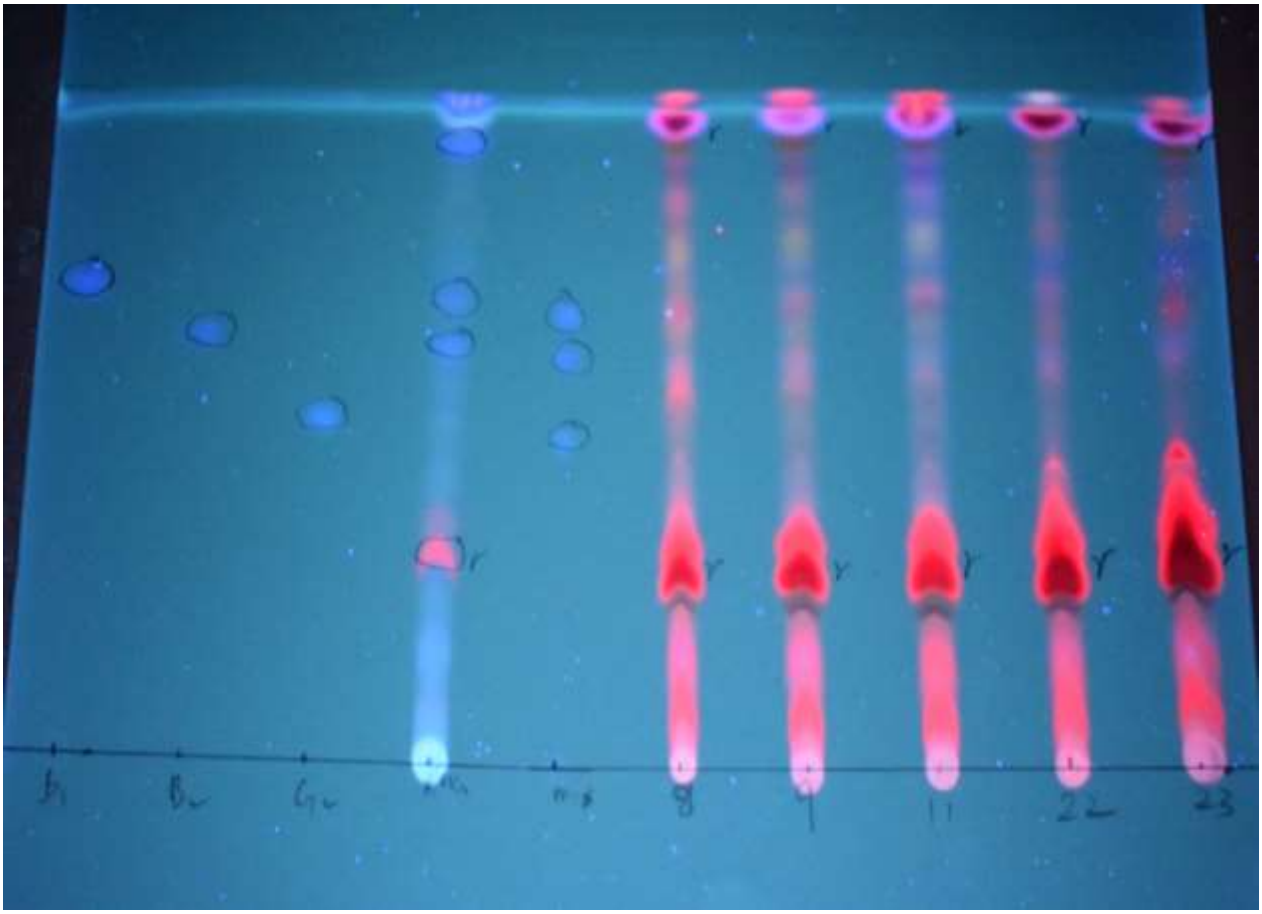

Figure 4.6: TLC plate with aflatoxin standards, chicken feeds and tea samples

The 8 black CTC tea samples in Figure 4.7, did not contain aflatoxin since there were no Retention Factor (RF) values of the tea samples corresponding to the RF values of the standards. On the other hand, the cow feeds which were used as an in-house positive control, had corresponding RF values to the standards, indicating positive presence of aflatoxin.

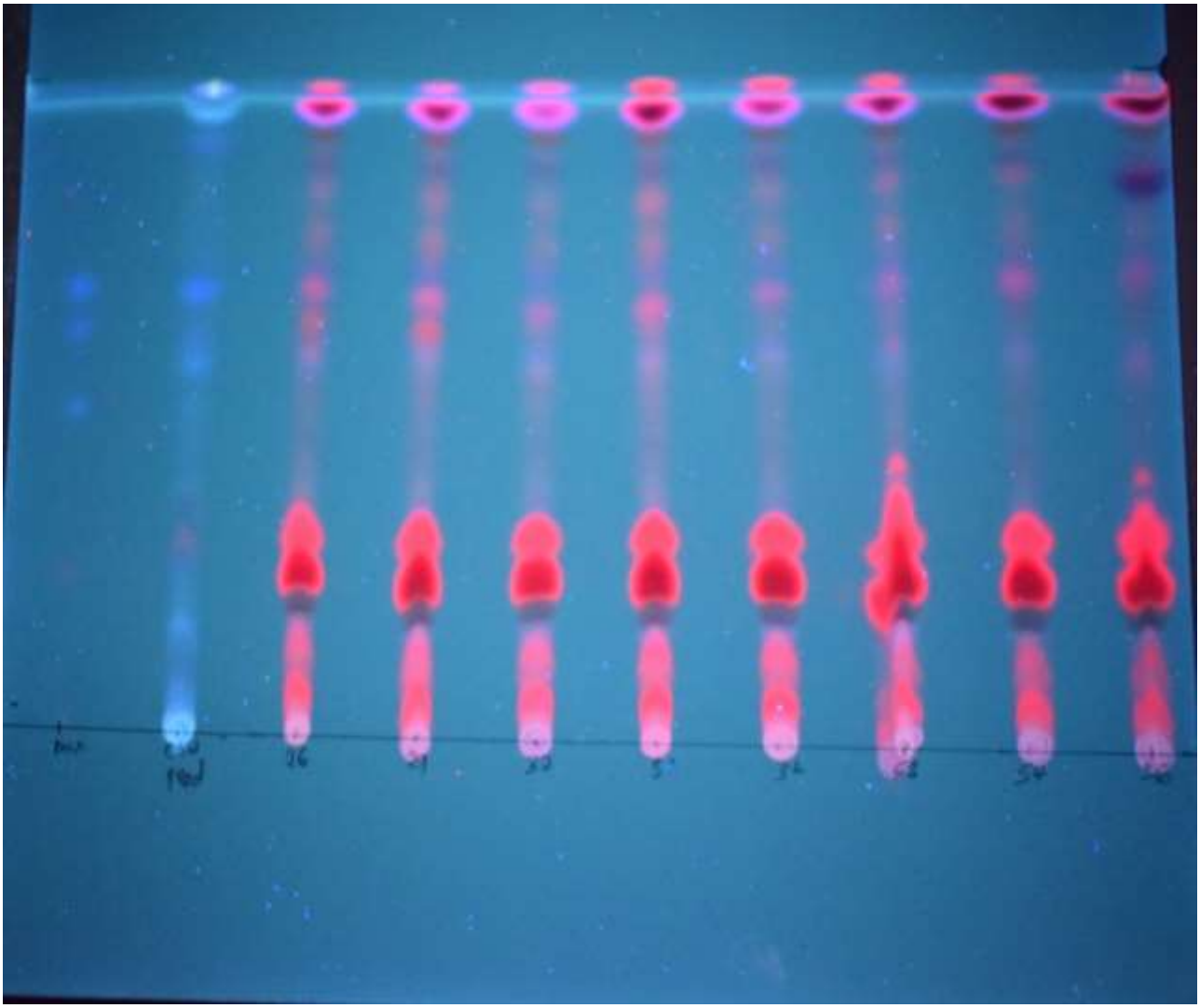

Figure 4.7: TLC plate mounted with a cocktail of standards, cow feeds and tea samples respectively.

As observed in the Figures 4.5, 4.6 and 4.7, which are all TLC plates mounted with the aflatoxin standards and tea samples, it is safe to conclude that of all the samples collected from Kenyan tea factories were aflatoxin-free in conformance to the set Uganda National Bureau of Standards, UNBS (2013) which suggested that the maximum content of total

aflatoxin in herbal tea products should not exceed 10µg/kg. This is because here were no RF values of the tea samples corresponding to the RF values of the standards. A confirmatory test was necessary and this was done using the HPLC method.

All the made black CTC samples that were previously found to contain yeasts and moulds were not to be contaminated with aflatoxin as observed in Table 4.12 and 4.13. It was therefore concluded that none of the made black CTC samples collected from the fourteen factories in the East and West of Rift Valley was contaminated with aflatoxin. This is because aflatoxin is usually a toxin that is produced by a mould known as *Aspergillus flavus* thus leading to screening of only those samples that were previously contaminated with moulds. From this study all the 23 made black CTC samples were mounted on TLC plates together with the known aflatoxin standards and the retention time together with fluorescing under a dark room was being observed. There was no blue or green fluorescing that occurred in any of the 23 made black CTC samples .Thus it was then concluded that aflatoxin B1, B2, G1 and G2 were absent in all the screened samples. This is in contrast to a previous study whereby tea samples were shown to be contaminated with mycotoxins including *fumonisins*, *deoxynivalenol*, and *enniatins* in recent concerns targeted at multi-mycotoxin analyses *(Sedova* et al., 2018).

Table 4.14:

Results of aflatoxin analysis using TLC in primary and secondary grades of black CTC made teas from selected KTDA tea factories in 4 regions in the East of Rift Valley.

Region	Factory	Grade	Presence of Aflatoxin
Region 1	Factory 1	F1	-ve
		D	-ve
	Factory 2	PF1	-ve
		D1	-ve
Region 2	Factory 2	F1	-ve
		Dust	-ve
Region 3	Factory 1	PD	-ve
		F1 (Mnf)	-ve
		F1 (Sacks)	-ve
		D (Mnf)	-ve
		D (Sacks)	-ve
Region 4	Factory 1	Fannings	-ve
		D	-ve
		DII	-ve
	Factory 2	F1	-ve

Regions 1 to 4 represent true regions of the KTDA factories. Factories 1 and 2 represent two selected coded factories from each of the 4 KTDA regions in the East of Rift Valley from which samples were drawn. BP-Broken Pekoe, PD-Pekoes Dust, PF- Pekoe Fanning, F-fanning, D-dust, FBD-fluid bed drier, Mnf-manufacture.DM-drier mouth.-ve-negative

Table 4.15:

Results of aflatoxin analysis using TLC in primary and secondary grades of black CTC made teas from selected KTDA tea factories in the West of Rift Valley

Region	Factory	Grade	Presence of Aflatoxin
Region 5	Factory 1	D	-ve
	Factory 2	F1	-ve
		D	-ve
		Drier mouth 1	-ve
		PF1 (bins)	-ve
		D (bins)	-ve
Region 7	Factory 2	PD	-ve
		DI	-ve

Regions 5 and 7 represent true regions of the KTDA factories. Factories 1 and 2 represent two selected coded factories from each of the 2 KTDA regions in the West of Rift Valley

from which samples were drawn.PD-Pekoes Dust, PF- Pekoe Fanning, F-fanning, D-dust, DM-Drier Mouth.

4.4.2 Aflatoxin Levels in Tea Samples Using High Performance Liquid Chromatography (HPLC)

A subsequent confirmatory test for aflatoxin was necessary following screening using TLC method. This was done using the HPLC method using the aflatoxin standards, in-house positive controls and a made black CTC tea sample. From Figures 4.8, 4.9, 4.10, 4.11 and 4.12 above, the various HPLC chromatograms showed peaks at various retention times except for Figure 4.13 whereby no peak was observed. This showed that the made black CTC tea sample was free from aflatoxin.

All the stored black made teas that were previously found to be contaminated with fungi, were found to be free from aflatoxin. The preliminary screening test was done using Thin Layer chromatography in comparison to aflatoxin standards and maize and ground nut samples suspected to be contaminated with aflatoxins. The confirmatory test was done using High Performance Liquid Chromatography. The maize and groundnut samples both contained aflatoxins whereas the made black tea samples were free from aflatoxins. Kenyan made black teas from the selected KTDA factories from the East and West of Rift are generally free from aflatoxins. 23 black CTC tea samples out of the collected 126 samples however contained yeast and moulds but within the accepted standards, that is, the CFU/g should not exceed 10^3 (ISO 21527-2).

Figures 4.8, 4.9 and 4.10 are chromatograms showing the various aflatoxin standards that have peaks at different retention ties. Different types of aflatoxins have different retention times depending on their molecular sizes which affects the rate at which they are eluted through the stationary phase during the high performance liquid chromatography.

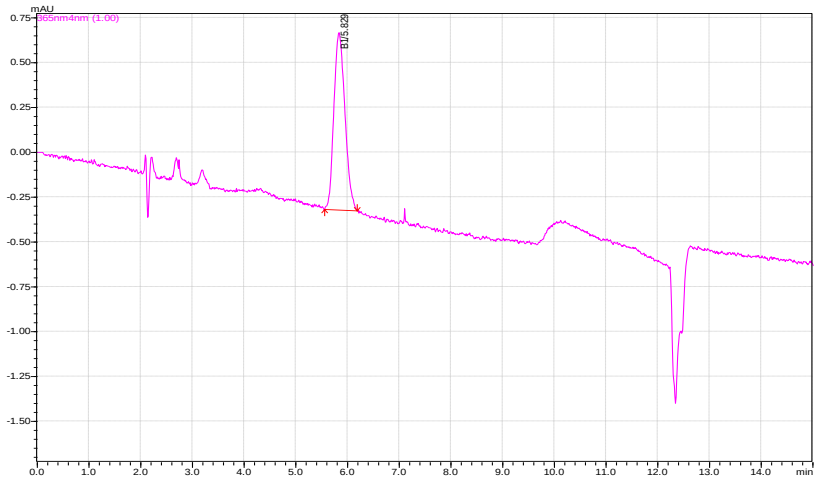

Figure 4.8: Aflatoxin B1 HPLC chromatogram

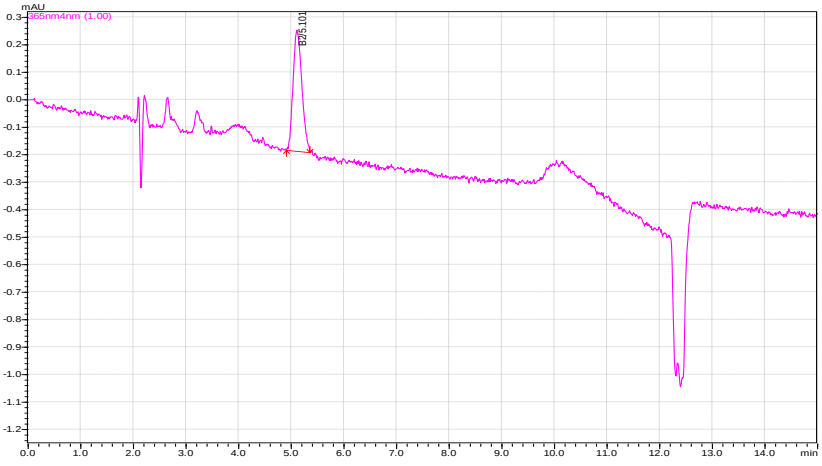

Figure 4.9: Aflatoxin B2 HPLC chromatogram

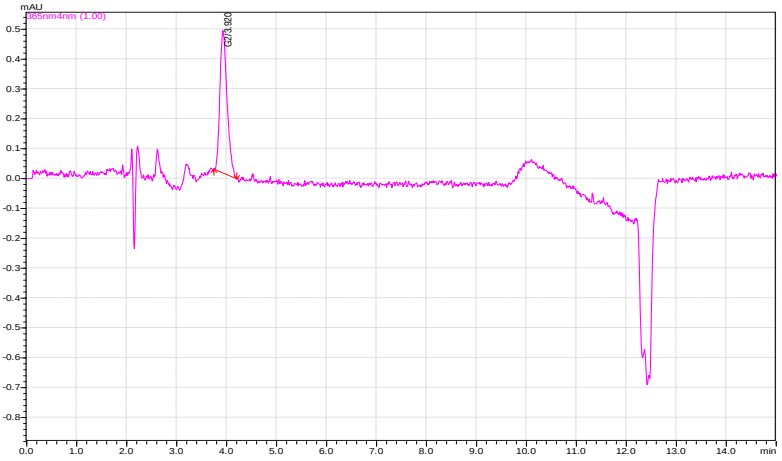

Figure 4.10: Aflatoxin G2 HPLC chromatogram

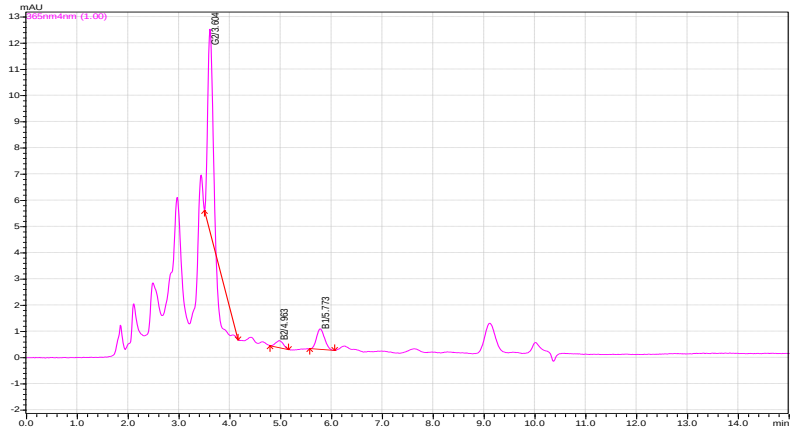

Figure 4.11: Maize sample HPLC chromatogram.

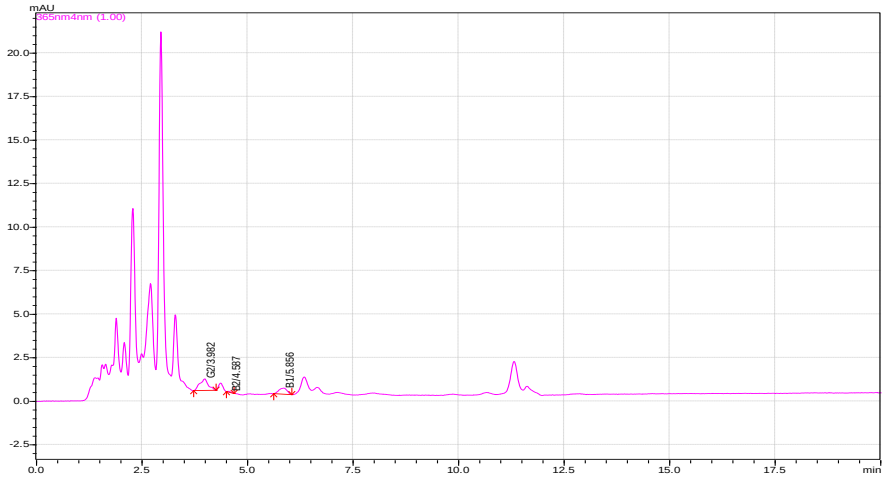

Figure 4.12: Ground nut sample HPLC chromatogram.

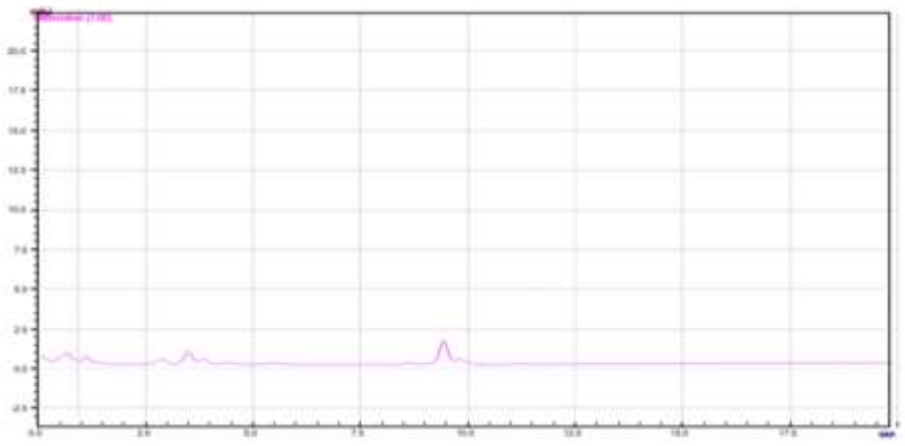

Figure 4.13: Made black CTC sample HPLC chromatogram.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Introduction

This chapter gives conclusions and recommendations based on the results obtained in chapter four. The conclusion highlights whether the Kenyan made teas conforms to the set national, regional and even international standards. The recommendations on the other hand, outlines the proposed mitigation methods to minimize contamination of the Kenyan made teas.

5.2 Summary of the Findings

This research study investigated microbial contamination, bacterial characterization, yeast and mould presence, heat-resistant bacteria, and aflatoxin profiling in Kenyan black CTC and green orthodox teas from selected Kenyan tea factories The study revealed differences in contamination levels between tea grades, regional variations in bacterial contamination, the influence of tea quality on auction prices, concerns about heat-resistant bacteria, and also the absence of aflatoxin contamination in the Kenyan made black CTC teas.

This research study comprehensively assessed the microbial quality of raw materials, in-process stages, and finished products of Kenyan black CTC and green teas from selected Kenyan tea factories, that is, a private factory and a multinational company factory Sampling and analysis were conducted to determine microbial contamination levels throughout the production process, ensuring compliance with Kenyan tea standards for

microbial safety along the tea value chain. Upon receipt at the factory, the tea leaves showed the highest levels of microbial contamination, primarily due to direct harvesting practices without prior cleaning. Contamination sources included soil microbes and those introduced during handling and transportation. Processing steps like steaming during green tea production and drying at 120 °C effectively reduced microbial populations. However, microbial reintroduction was observed during final sorting and packaging stages, particularly in the private tea factory where higher contamination levels were noted compared to a multinational company's facility.

It was noted during this study that there were consistent levels of bacterial contamination during withering, maceration, and fermentation stages, emphasizing the effectiveness of the drying process in eliminating bacteria. Notably, *Escherichia coli* and *Staphylococcus spp*. were isolated from samples, showing the need for stringent hygiene practices to meet microbial quality standards. *Salmonella spp*. were absent in the samples tested, suggesting compliance with safety thresholds in this regard. The findings clearly indicated the importance of maintaining rigorous hygiene protocols throughout tea processing to mitigate microbial contamination along the tea value chain.

Bacterial characterization was achieved by isolating and characterizing bacteria in processed Kenyan black CTC teas from selected Kenyan tea factories. Samples were analyzed before and after brewing as per manufacturer’s instructions to evaluate bacterial presence and conformity to microbial quality standards specified for Kenyan teas. This research study was able to unravel that the secondary tea grades (D-Dust and F-Fannings)

were generally more prone to microbial contamination compared to the primary tea grades which include BP-Broken Pekoe, PD-Pekoes Dust and PF- Pekoe Fanning. This could be attributed to the handling practices subjected to the teas. The primary grades were handled and packaged with more care and hygiene with strict observance to food hygiene measures compared to the secondary tea grades.

Bacterial contamination was generally higher in the sampled made black CTC tea grades from the West of Rift Valley compared to the East of Rift Valley except for the Dust grade. This could be attributed to better policies of food safety and hygiene being adhered to more strictly in the East of Rift Valley compared to the West of Rift Valley. It should be noted that the Made black CTC teas from the East of Rift Valley regions usually fetch way higher auction prices compared to the West of Rift Valley. This was mainly attributed to the tea quality as a result of observance of Good Agricultural Practices and Good Manufacturing Practices. It is therefore interesting to note that the East of Rift Valley made black CTC teas are generally less contaminated with microbes compared to their counterparts in the West of Rift Valley region.

This study also focused on bacterial characterization in processed Kenyan black CTC teas from selected factories, analyzing samples both before and post-brewing to assess conformity with microbial quality standards. Findings revealed that secondary tea grades (D-Dust and F-Fannings) exhibited higher susceptibility to microbial contamination compared to primary grades (BP-Broken Pekoe, PD-Pekoes Dust, PF-Pekoe Fanning), likely due to differential handling practices. Notably, bacterial contamination levels were

generally higher in black CTC teas from the West of Rift Valley compared to the East, except for the Dust grade, potentially linked to variations in adherence to food safety and hygiene policies between regions. This underscores the impact of Good Agricultural Practices and Good Manufacturing Practices in maintaining tea quality and reducing microbial risks.

This research study also revealed the existence of heat-resistant bacteria in the black CTC tea and this causes great concern as they may pose a health risk. Among them were some heat-resistant *E. coli* and heat-resistant *staphylococcus aureus*. However there was no heat-resistant *Salmonella spp.* identified. Consequently high levels of hygiene should be upheld in the affected factories to avoid cross-contamination of the made teas as these are disease-causing bacteria that can survive under high temperatures.

Furthermore, this research study was aimed at assessing levels of yeast and mould contamination in processed Kenyan black CTC teas from various KTDA factories in both the East and West regions of the Rift Valley, evaluating adherence to Kenyan Tea Standards (KS65). Across the East of Rift Valley factories, six out of eight sampled factories showed yeast and mould contamination in their teas, primarily affecting secondary grades like Dust (D) and Fannings (F). Notably, primary grades such as BP1 and PD generally exhibited lower contamination levels compared to secondary grads, reflecting differences in handling and packaging practices. The contamination pattern suggests that secondary grades, often stored in recycled sacks and handled less meticulously, are more susceptible to microbial colonization compared to primary grades

stored in new, sterile aluminium-lined brown bags. In contrast, the West Rift Valley factories showed varying contamination levels, with three out of six factories testing positive for yeast and moulds. Here, both primary and secondary grades were affected, underscoring potential lapses in hygiene practices and food safety protocols across regions. Nevertheless, the levels of contamination observed in all sampled regions remained below the permissible limits outlined in KS65, highlighting the overall compliance of Kenyan black CTC teas with set standards levels of yeast and moulds contamination of despite regional variations.

It was also noted in this research study that all the made black CTC samples that were previously found to contain yeasts and moulds were not contaminated with aflatoxin.

5.3 Conclusions

This research study on characterization of microorganisms in made black CTC and green teas from selected Kenyan tea factories, involved the isolation and identification of microbes in processed Kenyan black CTC and green teas from selected tea factories. They were systematically examined to assess conformity with the set microbial quality standards. The research was guided by specific objectives aimed at evaluating contamination levels, ensuring adherence to good manufacturing practices (GMPs) and proposing practical measures to enhance Kenyan made tea safety.

This study provides a comprehensive assessment of microbial quality in Kenyan black CTC and green teas across various stages of production in two distinct tea factories. The

research revealed that tea leaves upon arrival at the factory exhibited the highest microbial contamination levels, largely attributed to harvesting practices and transportation. However, processing stages such as steaming and drying effectively reduced microbial populations, highlighting their critical role in ensuring product safety. Nonetheless, microbial reintroduction during final processing and packaging was observed, particularly in the private factory compared to the multinational company's factory. Bacterial contaminants like *E. coli* and *S. aureus* were consistently present throughout processing showing the necessity for strict hygiene protocols. Importantly, the absence of *Salmonella spp*. in tested samples indicates some level of adherence to safety standards.

Boiling tea before consumption was identified as an effective measure to reduce microbial loads to safe levels, aligning with national microbial quality standards (KS65). Encouraging tea consumers to adopt this practice could significantly enhance public health outcomes by reducing exposure to potentially harmful microorganisms.

This investigation revealed varying levels of yeast and mould contamination across different KTDA factories in the East and West of the Rift Valley. Primary grades of tea generally exhibited lower contamination rates compared to secondary grades like Dust (D) and Fannings (F), highlighting the critical role of handling and storage practices in microbial control. This clearly emphasizes the importance of strict adherence to GMPs throughout the processing chain to minimize yeast and mould contamination.

It was also observed from this research study that Kenyan made black teas from the selected KTDA factories from the East and West of Rift Valley are generally free from aflatoxins.

Generally, this research study provided valuable insights into the microbial quality of Kenyan black CTC teas and offers practical recommendations to enhance manufacturing practices and consumer safety. By integrating suggested recommendations, KTDA and other stakeholders in the tea industry can effectively manage microbial risks, maintain product integrity and safeguard public health.

5.4 Recommendations

1. Establishment of a robust quality assurance program that includes frequent microbial testing of tea samples from each production batch should be done in the respective tea factories. This will ensure continuous monitoring of contamination levels and prompt corrective actions when standards are not met. Research and development initiatives should also be initiated by allocating resources for ongoing research into innovative technologies for microbial control in tea processing. New methods of storage and handling that mitigate contamination risks while ensuring product quality and compliance with regulatory standards should be explored. Minimizing human contact after the teas have been obtained from the drier mouth. This is because the research study clearly established that contaminant microbes were reintroduced at the point of sorting and packaging operations. This can be achieved by using machines like the vacuum pump lift during packaging as was observed to be in use in the multinational companies.

2. Packaging materials should be standardized across all grades of tea to ensure uniformity in storage conditions. Prioritize the use of new, sterile aluminium-lined brown bags for both primary and secondary tea grades to minimize bacterial contamination and maintain microbial integrity throughout the tea value chain. Education of tea consumers on the importance of boiling tea before consumption. The study confirms that boiling significantly reduces microbial contamination levels in black CTC teas, making it a crucial step for ensuring tea safety and quality before consumption.

3. The management of the Kenyan tea factories should facilitate knowledge exchange workshops among KTDA factories both regions to share best practices in tea processing and storage. Ultimately, this will encourage adoption of successful strategies that have proven effective in reducing fungal contamination in Kenyan made black CTC and green teas. Good manufacturing practices (GMPs) and appropriate hygiene should be adhered to for high quality and safe made black CTC teas for human consumption. This includes stringent sanitation protocols for equipment, storage areas, and packaging materials to minimize microbial contamination.

4. Incorporate non-invasive disinfection methods, such as using a vortex flow around UVC lamps in the final stages of tea processing. This technology can effectively sanitize tea surfaces and eliminate any residual microbes, ensuring compliance with microbial quality standards (KS65) and enhancing safety of the Kenyan made black CTC and green teas.

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5.5 Suggestions for Further Research

Further research should be done on:-

1. Characterization of heat-resistant microbes that survived after brewing and find out if they are pathogenic.
2. Non-Invasive disinfection of made teas in a vortex flow around UVC lamp as a method for sanitation.
3. Molecular characterization of microbes found in made black made CTC teas.

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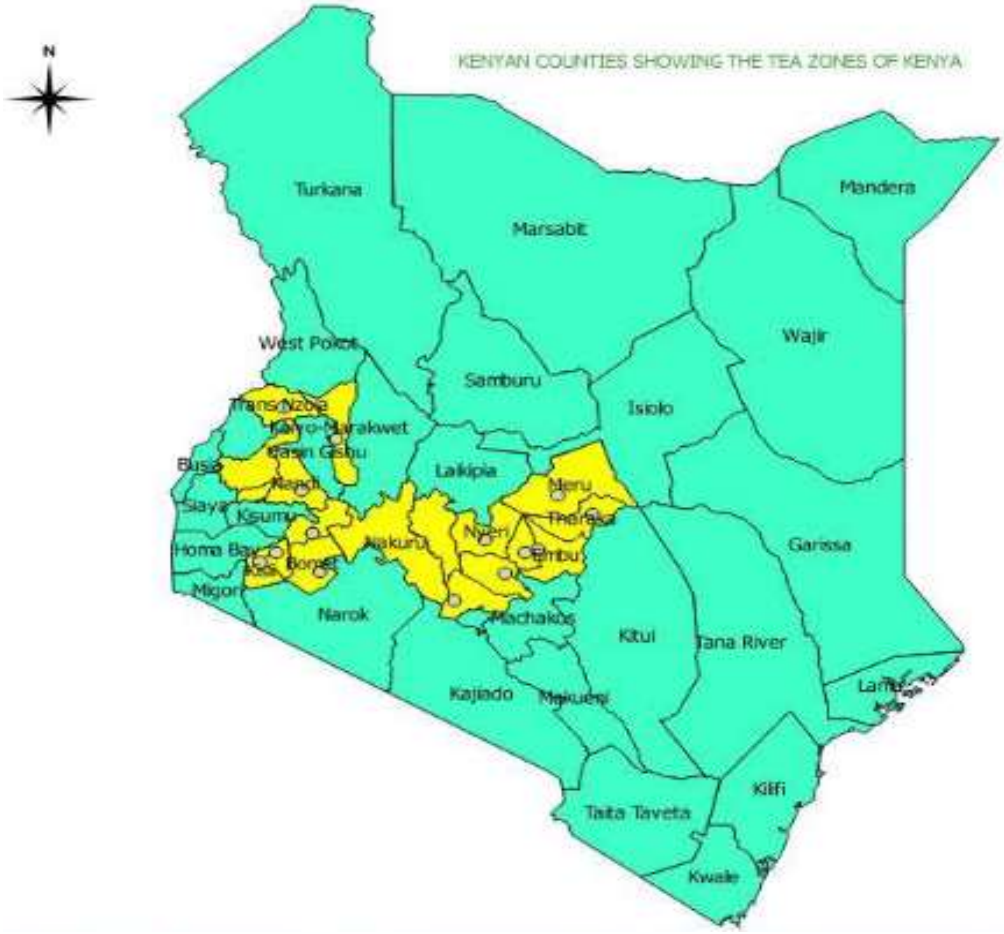
APPENDICES

Appendix I: Kenyan Map Showing the Tea Growing Regions in Kenya



Appendix II: A Map Showing the Spatial Distribution of Tea Production in Kenya

Figure: A map that is a clear representation of the spatial distribution of tea production in Kenya in the various tea growing counties.

Appendix III: Approval Letter from BGS

UNIVERSITY OF KABIANGA
ISO 9001:2015 CERTIFIED
OFFICE OF THE DIRECTOR, BOARD OF GRADUATE STUDIES

REF: PGC/MIC/003/17 **Date: 16TH NOVEMBER, 2022**

Mercy Cherotich,
Biological Sciences Department,
University of Kabianga,
P.O Box 2030- 20200,
KERICHO.

Dear Ms. Cherotich,

RE: CLEARANCE TO COMMENCE FIELD WORK/DATA COLLECTION

I am pleased to inform you that the Board of Graduate Studies has considered and approved your Masters research proposal entitled **"Characterization of Microorgnisms in Made Black Cut Tear and Curl (CTC) and Specialty Teas along the Tea Value Chain from Selected Factories"**. Subsequently the Board has also approved the following supervisors for appointments.

1. Dr. Simon O. Ochanda
2. Dr. Lyticia Ochola
3. Dr. Hellen Ogot

You may now proceed to commence field work/data collection on condition that you obtain a research permit from NACOSTI and /or an ethical review permit from a relevant ethics review board.

You are also required to publish one (1) article in a peer reviewed journal, with all your supervisors, before your oral defense of thesis.

You are required to submit through your supervisors, and HoD, progress reports every three months, to the Director, Board of Graduate Studies.

Please note that it is the policy of the University that you complete your studies within two years from the date of registration. Do not hesitate to consult this office in case of any difficulties encountered in the course of your studies.

I wish you all the best in your research and hope that your study will yield original contribution for the betterment of humanity.

Yours Sincerely,

Prof. J. K. Kibett
DIRECTOR, BOARD OF GRADUATE STUDIES.
JKK/hk

cc 1. Dean, SST
2. HOD, Biological Sciences
3. Supervisors

Appendix IV: NACOSTI License

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This is to Certify that Miss.. Mercy Cherotich of University of Kabianga, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Kericho on the topic: CHARACTERIZATION OF MICROORGANISMS IN MADE BLACK CUT TEAR AND CURL (CTC) AND SPECIALTY TEAS ALONG THE TEA VALUE CHAIN FROM SELECTED FACTORIES. for the period ending : 03/April/2025.

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Appendix V: Publication

Tea 43 (1) 2023, 17-40

EFFECTS OF PROCESSING AND HANDLING OPERATIONS ON MICROBIAL LOAD IN BLACK AND GREEN TEAS DURING TEA MANUFACTURE

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ABSTRACT

Tea (*Camellia sinensis*) is considered a low risk food in terms of microbial contamination because of the way it is processed, packaged and consumed. It is the most popular and widely consumed drink worldwide, only after water. However, there are possibilities of microbial contamination along the value chain and for this reason care should be taken to eliminate them. The study investigated microbial safety of tea along the processing line, providing insights into the diversity and quantity of microbes identified along the value chain. Tea processing steps are followed and microbial profile assessed from green leaf reception up to the finished product following stipulations in ISO Kenyan Black Tea Standard. This research study addressed the gap in existing research regarding the microbial status of teas across several processing stages in Kenyan tea factories, encompassing leaf reception, withering, maceration, oxidation, drying, sorting and grading, packaging and storage. The primary objective was to assess the microbial profile of teas along the processing line and recommend microbial quality control strategies aimed at minimizing cross-contamination risks during and after tea processing as per KS EAS 65:2018. By identifying and quantifying microbial populations at each processing stage, this study aimed to contribute valuable insights that could inform the implementation of effective Good Manufacturing Practices and hygiene protocols to ensure the production of microbiologically safe teas for consumers. This study focused on both cut, tear, and curl (CTC) teas free from microbial contamination. Bacteria and fungi were isolated using Nutrient Agar (NA) and Potato Dextrose Agar (PDA) respectively. *Escherichia coli* and *Staphylococcus spp.* were isolated of from tea, while *Salmonella spp*. was not detected. The yeast and mould detected were within the limits set in the standards. The findings show that even though there are set guidelines and standards for microbial control in tea processing like HACCP and KEBS the effectiveness of these control measures in various Kenyan tea factories is limited.

Key words: Tea quality, Processing, handling, microbes, GAP, GMP, Standards

INTRODUCTION

Tea (*Camellia sinensis, Theaceae*) is a widely consumed beverage on the globe, second only to water. This consumption is owed to its attractive aroma, refreshing taste and the potential health benefits it has [15]. These potential health