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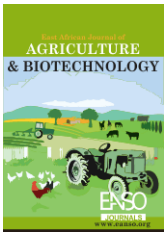

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Original Article

Effects of Processing and Handling Operations on Microbial Load in Black and Green Teas during Tea Manufacture

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Microbial Load, Tea Factories, Tea Processing, Nutrient Agar, Microbial Safety of Tea, Manufacturing Practices, Tea Value Chain, Potato Dextrose Agar, Black Tea, Green Tea.

ABSTRACT

Tea (*Camellia sinensis*) is considered a low-risk food in terms of microbial contamination due to the way it is processed, packaged, and consumed. It is the most popular and widely consumed drink worldwide, second only to 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 the 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 the microbial profile is assessed from green leaf reception up to the finished product, following stipulations in the 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 minimising 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 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.

APA CITATION

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INTRODUCTION

Tea is a widely consumed beverage globally after water because of its attractive aroma, refreshing taste, and potential health benefits [22]. These potential health benefits include: - antioxidant activity, ability to improve oral health [49], antibacterial [50], antifungal [50], and antiviral activities [12]. 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 [21], diabetes [44], vomiting [2], clastogenesis [31], inflammation [31], and cardiac-related issues [4]. However, in order to get these health benefits, consumers need to start by taking safe teas.

Kenya produces 22% of the world's black CTC (Cut, Tear and Curl) tea, placing it third after China and India in terms of tea production [26]. Kenyan tea is mainly exported to countries such as Pakistan, the United Kingdom, Egypt, Afghanistan, and Sudan, among others [51]. The export makes up a total of 95%, whereas the 5% is consumed locally. Tea as an agribusiness contributes up to 4% of Kenya's Gross Domestic Product, therefore 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 exchange [43]. The tea industry remains one of the profitable agro-enterprises in Kenya, in over 80 years of its existence, and is now the largest single produce export in the Country [35, 47]. As a whole, the tea industry is also a source of livelihood for 3 million Kenyans directly and indirectly along its value chain [8].

Tea is grown in regions with mountainous terrain where a favourable climate and rich soil are found. These regions are within the altitude range of 1500-1700 m above sea level and with red volcanic soil conditions that are favourable for tea production. Upon harvesting from the tea garden and before processing, tea leaves and buds are not washed or cleaned [23]. Storage conditions that are dry, cold, dark, and inert are critical for its preservation. Operations such as handling, packaging, and storage after drying may result in microbial contamination of the processed tea, even though the final drying stage at about 120°C is adequate to reduce and or eliminate any bacterial and fungal load [9, 48].

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 astringency [6]. After rolling, green tea leaves are not permitted to oxidise, and this is why they retain their light colour as well as flavour [18]. Unlike green tea, black tea processing involves withering and aeration to facilitate enzymatic oxidation of polyphenolic compounds and

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subsequent condensation resulting in the formation of theaflavins (TFs) as well as thearubigins (TRs) [3, 41, 52]; hence, they differ in their chemical composition.

Tea is best made by pouring hot water over processed dried tea leaves. However, green and white teas are preferably infused with sub-boiling water to preserve their flavour [5]. Water at sub-boiling temperatures may not eradicate all contaminants, including bacterial spores [40]. Contamination may also result in a reduction in quality and ultimately the demand for tea brands [14].

Aflatoxins and fungal colonies have been detected in teas due to contamination from tea plantations and unhygienic working conditions in some tea factories [30]. Fungi such as *Penicillium* are widespread and common during post-fermented tea storage and are vital in inhibiting the growth of spoilage or infectious organisms, and also give distinct aroma and quality to the teas [15, 20]. Despite processed tea being a potential host of microorganisms, many consumers around the world drink at least one cup of tea daily [23].

The tea exporting countries also demand safe teas for their consumers, as was seen from Kenya's top buyer, Pakistan in 2018, who dropped her sales by half, which was an equivalent of US$36.55 million loss in sales compared to the same time frame the year before as a result of requirement for rigorous aflatoxin tests [32].

Thus, this study investigated the level and type of microbial contaminants present in processed black and green teas in selected Kenyan tea factories. Even though its processing and consumption methods ensure the least microbial contamination, reports indicate that some Kenyan teas fail microbial quality tests [19]. In Kenya, little research work and documentation have been done on the microbial quality and safety of processed teas compared to the numerous works done on microbial plant and soil health, thus necessitating this study.

A previous research indicated the presence of spoilage and pathogenic microorganisms in Kenyan and Egyptian-made teas [19]. The presence of fungal strains in tea leaves and the presence of aflatoxin have been reported at various steps of tea processing and manufacturing [46]. The tea leaves are also potentially contaminated by environmental dust that settles on the different parts of the tea plant and could contain bacterial and mould spores [1]. *Staphylococcus aureus* can be transferred from humans to tea during the plucking, handling, and packaging operations [34].

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 a potential risk of pathogenic microbial contamination [1]. The emergence of harmful heat-resistant pathogenic microbes in other foods, such as beef, has been reported [7].

The current study investigated the microbial profile of black and green made CTC teas from green leaf reception, along the processing line to the finished product, from selected Kenyan tea factories.

Tea Standards

Made tea should meet certain physical, chemical, and microbial requirements as stipulated internationally, regionally, and nationally. To qualify for sale and export. Black and green teas must be processed, handled hygienically in accordance with ISO and East African Standards for green and black Teas (EAS 39).

Microbial Limits in Tea Standards

Food safety is an important component of food for human consumption. Microbial contamination in foods may cause spoilage and diseases to consumers and is of great concern as it endangers public health [17]. Their allowable quantity is given as colony forming units per gram (CFU/g) and should not

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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 in made tea.

General and Microbial Quality Control

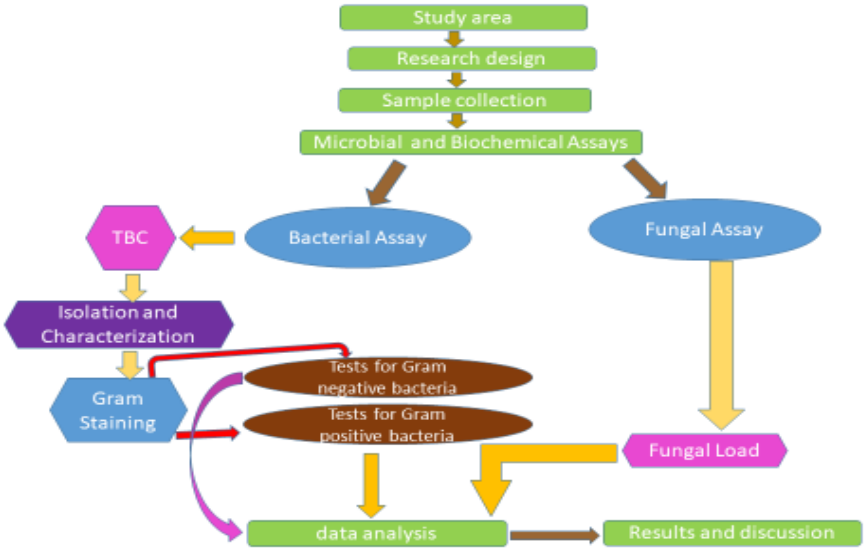
Kenyan tea industry standards are based on the International Organization for Standardization (ISO) and the Codex Alimentarius Commission (CAC) [33] standards of trade. Throughout the food supply chain, farmers, processors, and distributors must ensure that Good Agricultural Practices (GAPs), Good Manufacturing Practices (GMPs) are followed by producers (GMPs) and observance of safe handling and distribution as stipulated by the Tea Industry Code of Practice. The Kenya Bureau of Standards serves as the primary chain enabler and point of contact for the national codex within Kenya [33]. The Tea Directorate conducts ongoing enforcement audits of tea factories on tea regulation and guidelines, as well as aspects of good agricultural practices (GAPs), good manufacturing practices (GMPs), and best practices, as 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).

MATERIALS AND METHODS

Research Design

An experimental research design was used for this study.

Figure 1: Experimental Research Design Used in the Research Study



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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 soil conditions that are favourable for tea production. The tea growing regions cover an area of Latitude 1.3N to 1.3S and Longitude 38.4E to 34.3E.

Sampling

Two factories, a multinational and a private factory from the west of the rift processing green and black teas, were selected for the research. Tea samples were aseptically 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, while in process, tea was collected from withering, maceration, and fermentation just before the drying stage. The finished product was collected from the dryer mouth, sorted and graded, stored in the storage bins and after packaging, from various packaging containers. All the samples were aseptically handled to prevent cross-contamination.

Green and Black Tea Sample Collection

Green leaf, processed green and black CTC tea samples were collected from green leaf reception, withering, maceration (CTC), fermentation (CFU), dryer mouth, sorting and grading, storage bins, and packaged tea in containers. All the samples were aseptically handled to prevent any cross-contamination. Green Tea Processing Value Chain of Samples Sourced From a Multinational Company. 7 samples in three replicates were collected along a green tea processing line of a multinational company's tea factory. 15 samples with three replicates were collected along a black tea processing line of a multinational company's tea factory. 15 samples with three replicates were also collected along a black tea processing line of a private company tea factory. The total number of samples for the specific objective of this study was 37 samples, each replicated thrice during collection, including 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 was also collected. The finished product was collected just after drying, sorting and grading, and storage in the storage bins, and after packaging. They were then packed in zip lock bags to avoid contact with moisture during transportation in cooler boxes to the Tea Research Institute Miniature Factory for storage at room temperatures (22-24 ºC) and Relative humidity, awaiting analysis.

Microbial and Biochemical Assays

Total Bacterial Count

Total bacterial count (TBC) analysis was carried out according to International Organization for Standardization (ISO) Procedure (ISO 22000:2013) [11, 24, 38].

Isolation and Characterisation of Bacteria

This was done according to International Organization for Standardization (ISO) procedure (ISO 4833-1:2013) [25].

Gram Staining for Identification of Bacterial Isolates

Bacterial isolates were identified according to [13], where Gram-negative bacteria stained pink/red at the end of the Gram Stain, while Gram-positive bacteria stained purple.

Identification of Gram-Positive Bacteria

Mannitol Salt Agar (MSA)

This test was carried out according to Kateete *et al* [28]. 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

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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 [28].

Bile Esculin Agar (BEA)

This was performed using the method by Bullock & Aslanzadeh [10]. It was used in the 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 medium and caused light blackening of the medium [10]. This test was then followed by the catalase test.

Catalase Test

This test was in accordance with that of Bullock & Aslanzadeh, and it helped to distinguish between *staphylococci* and *streptococci* because they both cause blackening of the bile esculin agar medium. This was due to the possession of the catalase enzyme by *staphylococci* [10].

Sulfur Indole Motility Media (SIM)

The agar SIM was used to differentiate members of *Enterobacteriaceae*. *Staphylococci spp* were not motile [37], while on the other hand, *E. coli* and *Salmonella spp* were motile [36]. This test was carried out using the method used by Pollitt *et al*.

Identification of Gram-Negative (-ve) Bacteria

MacConkey Agar

This was carried out in accordance with Tanih *et al* [42]. Pink colonies on MacConkey media, which were indole positive, were considered positive for *E. coli* [42].

Simmon's Citrate Agar

The method used in this test was similar to that of Vaughn *et al* [45]. The pH indicator (bromthymol blue) changed colour from green to blue when the pH became alkaline. *Escherichia coli* were citrate negative [45].

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 were determined using ANOVA. The least significant differences Test (LSD) was used to separate the means. The probability limit was set at $p \leq 0.05$ significance level. The data and results were presented in the form of tables.

RESULTS AND DISCUSSION

At the point of reception of the tea leaves from the farm, the level of microbial contamination was highest, as shown in Table 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 or introduced during tea harvesting [1]. Microbial contamination may also be from the bags used to carry leaves during harvesting in the field, handling and inspection at leaf collection centres, loading into bags or crates at leaf collection centres and during transportation from transport vessels to the factories [19, 30]. During green tea processing, steaming is intended to stop oxidation, but it additionally helps in eliminating some of the microbes present in the tea leaves [39]. The drying process, at 120℃, is sufficient to eliminate all the disease-causing and most of the spoilage microorganisms present in the tea [29]. Subsequent handling and packaging operations after drying may reintroduce microbes

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into the finished product, as was observed in previous studies [1, 19, 23, 39]. A previous research indicated the presence of spoilage and pathogenic microorganisms in Kenyan and Egyptian-made teas [19].

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 [1], primarily due to direct harvesting practices without prior cleaning; tea leaves and buds are not washed or cleaned [23]. Contamination sources included soil microbes and those introduced during handling and transportation. Processing steps like steaming during green tea production and drying effectively reduced microbial populations [29, 39]. However, microbial reintroduction was observed during the 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 the withering, maceration, and fermentation stages, emphasising 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. *Staphylococcus aureus* could have been transferred from humans to teas during the plucking, handling, and packaging operations [34]. *Salmonella spp.* were absent in the samples tested, suggesting compliance with safety thresholds in this regard. The findings indicated the importance of maintaining rigorous hygiene protocols throughout tea processing to mitigate microbial contamination along the tea value chain. Exposure to microorganisms, which may be pathogenic, may occur during production and storage. Thus, during consumption, the infusion may be hazardous to one's health [48]. Thus, leading to possible interference with the tea made and ultimately may lead to a decline in the demand for tea brands that are affected [14].

Research on this domain helped to validate the safety of Kenyan teas and improve measures to control and eliminate contamination. This would enhance consumer confidence in Kenya's exports. It was anticipated that this form of marketing would make the Kenya 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. A sustainable and highly profitable tea sector would contribute to Kenya’s Vision 2030 [16].

In the current study, microbes were detected in packaged green tea as indicated in Table 1.

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Table 1: Total Bacterial Count of Microbes (CFU/g) Found in Green Tea Sourced from Factory X

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 the 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

Black CTC Tea

The level of microbial contamination was high in freshly plucked green tea leaves (Table 2) because the tea leaves were transported directly to the factory without cleaning or washing [23]. There was no variation in bacterial count of withered, macerated and oxidised tea. The drying process, at 120℃, was sufficient to eliminate all the bacteria that were present in the processed tea leaves [29, 39]. During the sorting step, bacteria were reintroduced to PD grade, whereas BP1 and PF1 grades were free from contamination. At the point of storage, awaiting packaging, the BP1, PF1, and secondary grades of teas were still not contaminated. This was mainly attributed to the high level of hygiene that was maintained at the multinational company's tea processing factory.

Table 2: Total Bacterial Count of Microbes (CFU/g) Found in the Black Tea Sourced from Factory B

Section	Leaf condition, status/ Tea Grade	Bacterial Count (CFU/g)
Transportation (from a 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	$>1.0x10^5$
Dry 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

Fresh plucked tea leaves from the farm had the highest level of microbial contamination (Table 3). This is because tea leaves collected on the farm are not cleaned or washed. Tea leaves microbial contamination occurred in the farm due to the introduction of soil microbes, during plucking or transportation, since the materials used for packing

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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°C, was sufficient to eliminate all the bacteria that were present in the processed tea leaves [29, 39]. During the sorting, bacteria were 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 a finding similar to that from previous studies [1, 19, 23, 39]. This was mainly attributed to the laxity in keeping a high level of hygiene at the private factory compared to that which was kept at the multinational company tea processing factory observed in Table 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.

Table 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	BP1 grade teas stored in bins	$2.3x10^4$
Storage Bin	PD grade tea stored in bins	$9.3x10^2$
Storage Bins	PF1 grade 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. These results were similar to a previous research that indicated the presence of spoilage and pathogenic microorganisms in Kenyan and Egyptian-made teas [1, 19]. Although most strains of *E. coli* are harmless, some strains could be harmful [27]. *Salmonella spp*. was not detected in the made tea samples. The results show that some teas did not meet the microbial quality standard requirements and there is a need to improve on handling the teas to avoid contamination in the factories identified, since there should be no *E. coli* and *Staphylococci spp,* just as seen from earlier [1, 19,23, 39]. This can be clearly seen in tables 4, 5 and 6, which constitute 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 tests.

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Table 4: Biochemical Characterisation of Microbes from the Black CTC Processing Line of Samples Sourced from A Private Factory.

Section	Leaf Condition/ Tea Grade	Selective Media	Biochemical Tests				
		BEA	XLD	MCA	MSA	SIM	SCA
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
	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
	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
Storage Bins	BP1 tea in the storage bin tea	no growth	large, flat, yellow colony	pink colony	yellow colony with yellow zone	Indole +ve motile, , no blackening	+ve
	PD tea in storage bin	no growth	large, flat, yellow colony	no growth	no growth	indole -ve motile, blackening	-ve
	Mixed secondary tea tea in storage buckets	no growth	no growth/red colony	no growth	no growth	indole –ve motile, blackening	-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 5: Microbial and Biochemical Characterisation of Microbes of Green Tea Samples Sourced from a Multinational Tea Company Factory

Section	Leaf condition/ tea grade	Selective media	Biochemical tests				
		BEA	XLD	MCA	MSA	SIM	SCA
Leaf Reception	Fresh Leaf	No growth	No growth	No growth	No growth	No growth	No growth
Enzyme inactivation	Enzyme inactivated leaf	No growth	No growth	No growth	No growth	No growth	No growth
Withering Troughs	Withered leaf	No growth	No growth	No growth	No growth	No growth	No growth
Maceration Cut Tear and Curl Machine (CTC 1)	Macerated leaf	No growth	No growth	No growth	yellow colony with yellow zone	motile, indole negative, blackening	Negative
Maceration CTC 2	Macerated leaf	No growth	No growth	No growth	No growth	No growth	No growth
Maceration CTC 3	Macerated leaf	No growth	No growth	No growth	No growth	No growth	No growth
Dryer Mouth	Processed leaf						
Packaging	Packaged leaf	no growth	large, flat, yellow colony	pink colony	no growth	No growth	Negative

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.

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Table 6: Microbial and Biochemical Characterisation of Microbes of Black Tea Samples Sourced from a Multinational Tea Company Factory.

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

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.

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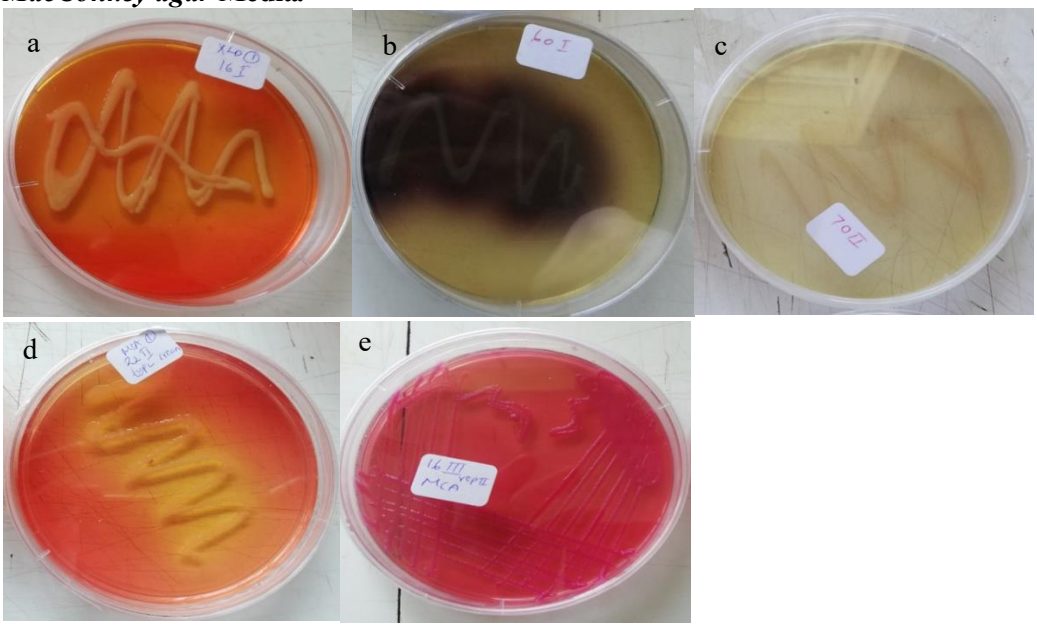
Growth of Bacterial Colonies on Selective Media

Figure 2: Plate Showing the Growth Characteristics of [a] *E. coli on Xylose Lysine Deoxycholate (XLD)* Media, [b] *Staphylococcus aureus* on *Bile Esculin Agar (BEA)* Media, [c] *E.coli on Bile Esculin Agar (BEA)* Media, [d] *Staphylococcus aureus on mannitol salt Agar (MSA)* Media, [e] *E.coli on MacConkey agar* Media.



From Figure 2 above, 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 a, 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. From Plate b, *Staphylococcus aureus* had good growth in Bile Esculin Agar (BEA) media and caused light blackening of the media [9]. *E. coli* had good growth in BEA as shown in Plate c, but with no blackening of the media. Both *Staphylococcus aureus* and *E.coli* were found in some of the black made CTC samples in this research study, as shown on Plates b and c. This showed that some of the tea samples were not completely safe for human consumption as they 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°C so that the pathogenic microbes can be eliminated. *Staphylococcus aureus* used mannitol as a source of food and produced acidic fermentation by-products, which reduced the mannitol salt media. The pH indicator, phenol red, turned yellow when the media became acidic, as was observed in Plate D. *Staphylococcus aureus* fermented mannitol and grew as yellow colonies with yellow zones [28]. This further confirmed the presence of *Staphylococcus aureus* in the made black CTC samples that were 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 drying machine. This is because, from this study, it was discovered that the teas obtained from the drying step of processing were completely sterile and that microbial contamination was reintroduced during the sorting and packaging operations, which involved human

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handling. Pink colonies on MacConkey agar media, which were indole positive, were considered positive for *E. coli* [42]. 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 pink-red colour as observed in Plate 5. This was further confirmation that the collected Kenyan-made black CTC samples were contaminated with *E. coli*. *E. coli* was the most common microbe isolated during the research study. Although most strains of *E. coli* are harmless, some strains could be harmful [27]. The study did not unravel which particular strains the isolated *E. coli* belonged to due to limited resources.

CONCLUSION AND RECOMMENDATIONS

This study provides a comprehensive assessment of microbial quality in Kenyan black CTC and green teas. Tea 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. Bacterial contaminants like *E. coli* and *S. aureus* were consistently present throughout processing, showing the necessity for strict hygiene protocols. The findings of this study were similar to previous studies where Bacteria, yeast, mould, and coliform were observed before and after boiling in all the studied samples [23].

Importantly, the absence of *Salmonella spp*. in tested samples indicates some level of adherence to safety standards. Establishment of a robust quality assurance programme will ensure continuous monitoring of contamination levels and prompt corrective actions when standards are not met. The findings clearly indicated the importance of maintaining rigorous hygiene protocols throughout tea processing to mitigate microbial contamination along the tea value chain.

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