ASSESSING MICROBIAL QUALITY OF BEER HEAT TREATED BY FLASH AND TUNNEL PASTEURIZATION.

KATAKALA M SIAKAVUBA

A DISSERTATION SUBMITTED IN FULFILMENT FOR THE MASTER'S DEGREE IN APPLIED FOOD MICROBIOLOGY AT THE UNIVERSITY OF ZAMBIA.

UNIVERSITY OF ZAMBIA

DECLARATION

I, Katakala Muyoba Siakavuba, declare that this master thesis represents my own work. It has not previously been submitted for a postgraduate degree or any award at the University of Zambia or any other institution. All cited works and materials from other sources have duly been acknowledged and references thereby given.

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CERTIFICATE OF APPROVAL

This dissertation submitted by Katakala Muyoba Siakavuba is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Applied Food Microbiology at the University of Zambia.

Supervisors

Name:……..……

Signature:

……Date……..…..

Co-Supervisor

Name:……...……

Signature:…….*Date*……

……..

Name and Signature of Examiners

Internal Examiners

1. Name: ……..……Signature: …….……..

Date: ……..……

2. Name: …….……..…Signature: ……

Date: ……...……

3. Name: ……..…Signature: ……..

Date: ……...……

ABSTRACT

Beer is mainly produced through fermentation. This process happens in the presence of the only micro-organism that is allowed which is *Saccharomyces cerevisiae* which ferments the small sugars to produce alcohol, carbon dioxide and energy. A large percentage of microbial contamination occurs when the yeast strains are introduced into the process. Beer is a high nutrient product that is susceptible to microbial contamination. Beer contamination is when microorganisms are present in the product. These microbes are classified as beer spoilage microorganisms, aerobic bacteria and wild yeast which have different effects on the quality of beer. Beer spoilage microorganisms are responsible for the staling of beer which is also a consequence of aerobic bacteria present in the beer. Presence of wild yeast in the beer always gives the beer a cloudy color and causes what is termed as haze. This microbial contamination is only able to be controlled through pasteurization which is the heat treatment of beer at the end of beer production. There exist three (3) types of pasteurization across the globe. Tunnel pasteurization, flash pasteurization and sterilization. But in Zambia, only two (2) of these are used. Tunnel pasteurization and flash pasteurization are the main pasteurization processes that most macro and micro breweries use. Comparison of the effectiveness of flash and tunnel pasteurization was studied in this research.

Twenty-four samples were collected and analysed. Different agars were used to inoculate these samples depending on the type of microbes that were needed to be identified in wort stage, fermentation stage and before and after the pasteurization samples. Biochemical tests were done to further identify the variety of microorganisms that were present. The micro-organisms associated with beer were identified, the various types of micro-organisms were also identified and the comparison between flash and tunnel was assessed through the microbial analysis and serial dilutions that were done.

Results show that there were traces of beer spoilage bacteria and wild yeast. This contamination by microorganisms is a clear indication of insufficient/no cleaning of vessels. Recommendation is to implement CIPs of the dilution system of the brewery, CIPs of the BBTs to Filler to be started, hose management at filtration to be identified by developing an SOP for it.

DEDICATION

This work is dedicated to my sister Sianjange Siakavuba Mulaisho for supporting me during my early academic journey. My late sisters Muleya Zuka Siakavuba and Ruth Museba (MTSCRIP) who's absence in my life has been a source of encouragement and strength. My parents Dr B.J Siakavuba and Mbaita Brenda Siakavuba for teaching me hard work and discipline during my early childhood. My beautiful daughter Khetiwe Nyirenda whom I work extremely hard for.

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ACRONYMS AND ABBREVIATIONS

AAB – Aerobic Acetic Acid Bacteria

AB – Aerobic Bacteria

ABV- Alcohol By Volume

BA – Biogenic Amines

BBT – Bright Beer Tank

BS – Beer Spoiler

CIP -Cleaning In Place

CV - Crystal Violet

DMS – Dimethylsulphide

FV – Fermentation Vessel

KB- Kegged Beer

KL - Copper sulphate according to LIN

KOH – Pottassium Oxide

LAB – Lactic Acid Bacteria

LWYM – LIN's wild yeast detection media

MYGP – Yeast and Mould-copper sulphate agar

NBB - Nachweismedium für Bierschädliche Bakterien

PB – Pasteurised Bottle

PCA – Plate Count Agar

PK – Pasteurised Keg

PU – Pasteurisation Units

SDM – Swarz Differential Medium

SPP – Specie/ Species

SSSB - Swing Stopper Sample Bottle or Schott

SV – Storage Vessel

TM – MYGP-copper sulphate agar according to Taylor and Marsh

WLN - Wallerstein Laboratories Nutrient Media

WY- Wild Yeast

YM – Yeast and Mould Media

CHAPTER 1

1.1. Introduction

Beer brewing is an ancient concept that has changed as the years have gone by. Beer is an alcoholic beverage or drink made from yeast, water, malted barley, maize, cassava, or sorghum and flavoured with hops (Learners, n.d.). Brewing was recorded in the early 7000BC in China and later Europeans copied it and they discovered they could add hops in the brewing process (Rainbow, 1981).

In beer production, there are a lot of raw materials that are used. These include barley, malted barley, cassava, maize, sorghum, rice, and wheat. These different raw materials create complex flavours based on the different brand profiles. Beer production, or alcohol production, is the breaking down of complex sugars into simple fermentable sugars and being exposed to yeast for a period of time to produce carbon dioxide and alcohol. With the chemical equation being.

$$C_6H_{12}O_{6\ (aq)} \longrightarrow 2C_2H_5OH_{(aq)} + 2CO_{2(g)} + 2ATP$$

$$\text{Glucose} \longrightarrow \text{Alcohol} + \text{Carbon dioxide} + \text{energy}$$

Opaque beer production is widely practiced in countries like South Africa, Uganda, Zimbabwe, Zambia, Malawi and Tanzania (Okambawa, 2011). A starch source (sometimes malt), water, and yeast are used in the alcoholic fermentation process to create opaque beer, a weak alcoholic beverage. This is an alcoholic beverage that is produced by a few African countries through fermentation. Most populations of African countries particularly those living in rural areas, typically drink a lot of opaque beer because they view it as both an alcoholic beverage and food (Tiisekwa, 1986). Opaque beer fermentations happen whilst in the carton and already packaged for the consumer. If not consumed within 6 days of packaging, the alcohol content can get to 6% ABV. In a few countries that produce beer at small scale, it is produced in small drums where water and maize, malted sorghum, malted barley, cassava, yeast and water are put in a drum and allowed to ferment and form bubbles, hygiene is less considered. For breweries that produce beer on a large scale, hygiene is considered to prevent economical downfall. Beer as earlier mentioned is highly susceptible to microbial contamination during the production process. For fermented beverages like the local munkoyo which Zambians make at small scale, hygiene during the making is rarely considered.

There different types of alcoholic beverages that are produced across the globe. From wine brewing, brewing of rum, whisky, gin, vodka, opaque and clear beer. In all these production processes, fermentation occurs (Quain, 2002).

As mentioned earlier, the entire process is prone to microbial contamination. These contaminants could be aflatoxins, wild yeast or bacteria (Priest, 1996). More research is still being done to discover whether there is ever viral contamination in the brewing industry. The most common type of microorganisms are the beer spoilage micro-organisms which are mainly bacteria. The only micro-organism allowed during the production of opaque, traditional and clear beer is the yeast that begins the fermentation. It breaks down the sugars to produce alcohol and carbondioxide. The alcohol percentage differs for the production of different beers. Munkoyo however is not an alcoholic beverage but if allowed to ferment for a longer period of time, it becomes alcoholic (Schoustra, 2019).

In the production of beer, because of the nutrient rich environment, the entire process is susceptible to micro-organisms. The yeast used is the Saccharomyces species of yeast that's allowed during the production of beer. Any other micro-organism that's used is said to be a contaminant and is called wild yeast. A few micro-organisms are associated with brewery contamination like Lactobacillus, Pediococcus, Megashaera, Pectinatus and Entarobacteiaceae (Back, 2009). These are mostly associated with insufficient cleaning of vessels or brewing equipment, failure to achieve boiling times, failure to sterilize the oxygen or air that's being used. Opaque beer production is different from other types of brewing. Most breweries use malted sorghum instead of malted barley, it is sour in taste because its flavoured with lactic acid and hops are not added. Hops are the first defence for the brewery to fight against microbial properties because they have antibacterial properties and it's for this reason that most breweries use hops and opaque beer is prone to contamination by microbes (Campbell, 1996). Also, the yeast remains in the carton and fermentation occurs in the carton after its already been packaged.

Hygiene in a brewery is important because it prevents contamination which could consequently affect the quality of beer. In opaque beer production where pasteurisation occurs before fermentation, off-flavours are mostly observed. This is said to be contaminated and there many off-flavours that are observed.

The aim of this research was to identify areas where microbial contamination occurs, the intensity of the contamination and use the last defense of eliminating the contamination using flash or tunnel pasteurization with the aim of also comparing the two methods of pasteurization. Results from this research will provide an in-depth understanding of the differences and efficiencies of each method and compare the efficiencies and advantages to what happens in the actual brewery. This gives the brewery ways in which to control, mitigate, and eliminate microbial contamination. The detection and characterization of beer microorganisms will be discussed in this research.

1.2. Statement of the problem

Pastuerization is used in the improvement of the biological stability of the final product, in this particular case, beer. According to a study reported by (Anon., 2012) beer pasteurization and freshness are challenges faced by beer manufacturers. The largest beer making company accounts for 80 percent of U.S. sales and its target loss is 5% with the largest losses of 3.7% (Anon., 2012) going to freshness which is a result of microbial contamination. . The next two top companies account for a combined 10 percent of sales and target loss of 7% with consumer returns of 4.9% (Anon., 2012), bringing the top four total to 90 percent of the U.S. market. In this report, it also states that sales are affected by off-flavours which are recorded and are a sign of microbial contamination. In Africa, brewing microbial contamination is a major cause of sales for most breweries. For Opaque beer production, a research is being done on the sterilization of opaque beer with the use of *Moringa oleifera* (Ayirezang, 2016). No other research has been done other than the usual pasteurization. In Zambia, the current pasteurization systems in place are proving to be insufficient. Many off-flavours have been observed on the market which are describes as buttery, sour, acetic, vinegar and DMS. All these off-flavours point to microbial contamination in the brewery that are controlled and moderated through pasteurization which is proving to be insufficient. This microbial contamination could result in sickness and in extreme cases cause death due to the release of biofilms. There have been cases of contaminations in the brewery after pasteurisation and this research will assess the effectiveness of the two types of pasteurisations at a brewery in the city of Lusaka. Many variables will be looked at during this research to identify the points at which beer is contaminated and also the effect of the contamination if the pasteurisation is not as effective as it should be.

1.3. Significance of Study

This study will help beer producing companies improve freshness scores of the beer. It will generate information which will at a later stage improve their methods of pasteurization to ensure that the consumer has fresh and high quality beer. Breweries of both opaque and clear beer need to comply to proper brewery hygiene and beer safety as this results in production of high and good quality beer. This will prevent breweries from decanting the beer which is contaminated as this will prevent penalties by the environmental councils, consumer organisations and the economic loss. This will allow the brewery to always ensure that CIPs will always be effective and allow them to resolve all potential microbial contamination points.

1.4. Aim

The aim of the study was to assess the effectiveness of flash and tunnel heat treatment on microbial quality and quantity in processed beer.

1.5. Specific Objectives

The specific objectives were to:

1. Isolate and identify microorganisms associated with packaged beer contamination
2. Determine the microbial diversity and quantity of processed beer.
3. Assess the effectiveness of the flash and tunnel pasteurization on microbes present before pasteurisation by comparing the two methods being used.

1.6. Research Questions

1. What type of microorganisms are associated with beer contamination at the brewery?
2. What is the microbial quality and quantity of raw packaged beer?
3. How effective is flash and tunnel pasteurisation?

CHAPTER 2

2.1 LITERATURE REVIEW

2.1.1 Beer Production

In the production of clear and opaque beer, ingredients are the same. Most breweries in Zambia use malted barley, maize, cassava, water and sorghum. Whilst in Europe the major ingredients are barley, hops and water with an obvious ingredient called yeast. Different companies have different recipes and that's what they include. According to an article written by (Encyclopedia, October 2023) it states the different ingredients used in the beer production companies. For example, Japan uses mostly Rice for the production of beer. The wort is made from malted barley, unmalted wheat, and aged hops. In this article, it states and shows which beers are produced where, what they use and what type of beer is produced there.

Farmers and brewers work together extremely well, and their combined influence is worldwide. In order to guarantee the production of high-quality beer, brewers rely heavily on farmers for a steady supply of premium grains and hops (W, 2020). For many families across the nation, this is a source of revenue. Furthermore, it yields revenue for the nation, which is allocated towards its development.

In beer production, the malt is milled to expose the endo-sperm where enzymes are present to break the long carbohydrates chains (Quain, 2002) to fermentable sugars. The sugars are then filtered to be collected in wort and finally cooled and pitched with brewers yeast. In the production of opaque beer, ingredients are the same as those used in the production of clear beer. In most cases, ingredients include malted barley, sorghum and maize. According to a review on opaque beer by Werner Embashu (2019) from Namibia, *Omalovu* which is a popular traditional opaque beer in Namibia which uses Malted Sorghum or pearl millet grown in prevailing semi- and conditions. In the review (Embashu, 2018), also stated that it is heterogenous and unpasteurized alcoholic beer.

Beer resists microbial contamination due to the presence of inhibitors such as hop compounds, alcohol, carbon dioxide, and sulphur dioxide, as well as low pH, lack of nutrients, and oxygen (Quain, 2002). Furthermore, reduction of contamination can be achieved through low-temperature storage, filtration, and potential pasteurisation.

Typically, microorganisms do not grow in beer. Low fermentation, high oxygen content, low pH, low hop concentration, and low alcohol content beers are the harsh conditions which are prohibitive to the growth of microorganism. According to (Rainbow, 1978), (Calina C, 2022), in a journal entitled Beer Safety started that organisms that can grow in beer after extended exposure are also considered potential beer spoilers.

2.1.2 Microbiology of Beer Production

Microorganisms that cause brewery products to spoil are known as brewery contaminants. Breweries

encounter several significant challenges, one of which is the production and preservation of beer quality. Beer quality characteristics are deteriorating due to spoilage microorganisms attacking the beers produced by breweries (Rainbow, 1978). Because spoiling organisms can enter the brewing process at any time, even before the product is dispensed, the microbiological stability of the finished product may be jeopardised from very early on in its production. Microorganisms can infect brewing raw materials like malt, hops, and occasionally brewing water. These must be eliminated during the brewing process to keep the wort and beer from spoiling.

Bacteria cells are the most common microorganisms found on earth. They are also common in and breweries across the globe. According to (Rainbow, 1981), bacteria including *lactic acid bacteria, acetic acid*, *Enterobacteriacea, Pectinatus, Megasphaera*, and wild yeast are among the microorganisms that cause beer to spoil. Ensuring a high degree of cleanliness requires effective quality assurance procedures, particularly in situations where sterile filtration of beer or bottle/can pasteurisation is not present. Despite recent significant improvements in overall brewery hygiene, secondary contaminations are now responsible for a growing percentage of microbiologically spoiled beers. A brewer may produce beer with a longer shelf life, fewer customer complaints, higher production rates, and lower rejection rates by sampling for cleanliness and raising awareness of hygienic conditions.

Although indirect beer spoiling organisms do not grow in finished beer, they may begin to do so at certain points during the process, which could lead to off flavors in the finished product. They usually appear in the pitching yeast or early in the fermentation process, leading to quality flaws that need to be prevented by blending. This category includes some Saccharomyces spp. wild yeasts, enterobacteria, and some aerobic yeasts, according to (Back, 1994).

Indicator organisms are microorganisms that are found in water bodies and are used as a stand-in for pathogens in the environment. These organisms do not initiate spoilage; rather, they arise due to inadequate cleaning or mistakes made during production. They are frequently linked to the growth of organisms that cause beer to deteriorate. Representatives of this group include aerobic wild yeasts and *Acetobacte*r spp (Back, 2009).

Latent organisms are microscopic microorganisms that are sometimes discovered during the brewing process (Back, 2009). Under some circumstances, they can withstand several stages of the process and become independent of the finished beer ((Harper 1981, n.d.). The presence of these organisms in the brewery is often the consequence of internal construction projects or contaminated process water. Members of this group are typically common soil and water organisms. However, if found frequently, they should be interpreted as a sign of poor hygiene. Common latent microorganisms found in the brewery include spore-forming bacteria, enterobacteria, and a few yeast species.

2.2 Sources of Contamination

Primary contaminations in the brewery typically come from the yeast, wort, fermentation, maturation, or pressure tanks; secondary contaminations typically come from the bottling, canning, or kegging processes. Although secondary contaminations in the bottling area are responsible for about half of microbiological issues (Back, 1997), primary contaminations can have far more severe and widespread effects. While indirect spoiling organisms are primarily primary contaminants, absolute beer spoilage organisms can appear at any point during the process. Depending on where in the process an organism is found, determines its spoiling character. The brewing yeast should also be considered a contaminant after filtration (Haikara, 1984).

Because spoiling organisms can enter the brewing process at any time, even during the dispense process, the microbiological stability of the finished product may be jeopardized from very early on in its production (Bamforth, 2009). Beer may be contaminated by microorganisms that come from different sources. While secondary contaminants are added to the beer during bottling, canning, or kegging, primary contaminants come from the raw materials and the brew house vessels. Although secondary contaminations account for about half of the microbiological issues that have been documented, primary contaminations can have more disastrous effects, including the loss of an entire brew (Vaughan, 2005). Raw ingredients and/or dirty brewing equipment are the main sources of potential contaminants in beer. In order to keep wort and beer from spoiling, microorganisms that may have infected brewing raw materials like malt, hops, and occasionally brewing water must be eliminated during the brewing process (Bamforth, 2009).

2.2.1 Malt

Malt is cereal grain that has germinated and been dried. Soaking the grain in water causes it to germinate, and drying it with hot air prevents it from germinating any more (Vaughan, 2005). There are several ways that growing, storing, or malted barley can become contaminated. Reduction in gas stability or gushing—the uncontrollably ejecting of beer from its container—is the most well-known consequence of the microbiota of barley and malt. While many different fungi have been linked to gushing, *F. moniliforme* and *F. graminearum* are the most well-known. *Fusarium, Nigrospora*, and *Trichoderma* strains have been found to contain small fungal proteins called hydrophobins in their cell walls (Priest, 1996).

2.2.2 Water

Brewing and the quality of the water are closely related. (Bamforth, 2009). Water makes up to 92% of beer (Briggs, 2004). Therefore, it needs to be free of any contaminating organisms as well as other impurities like organic materials and minerals. Hill and Bamforth (2009) mentioned that not all materials fit for ingestion can be used to make beer. The introduction of spoilage organisms from water introduced after fermentation, such as during beer dilution after high gravity brewing or from vessels rinsed with contaminated water, is the primary microbiological

concern. (Bamforth, 2009). Any type of microbial contamination, such as the presence of coliforms, should be eliminated from water as this can result in off flavours in beer.

2.2.3 Pitching Yeast

A single cell fungus is the only microorganism allowed in brewing (Quain, 2001). This microorganism converts sugars to alcohols and carbon dioxide. These exist and work well in the the form of millions of cells. These yeast strains are *Saccharomyces pastorinus* for lagers and *S. cerevisicae* for ales (Back, 2009). The predominant micro bacterial contaminant in a larger or ale brewery is the pitching yeast, which is capable of contaminating beer from fermentation to fermentation and vessel to vessel. If there is contamination of the pitching yeast, the quality which compromises of taste and consistency of the beer is compromised leading to production of a beer that is outside the specifications of the brand manual of the beer (Bamforth, 2009). Controlling the microbiological state of the pitching yeast begins from the early stages of yeast growth which in the brewery was called "Yeast propagation". Yeast propagation allows for the growth of yeast without any microbes meaning it should be inoculated aseptically into the wort. Yeast propagation from the brewery uses a powder which is inoculated at lab stage into a conical flask and later transferred into a Carlsberg flask. The last stage of yeast propagation is done in what the brewery called yeast propagation tank where wort and a few million cells are in continuous circulation and aeration to allow the growth of yeast for the brewery to use over a certain period of time.

2.2.4 Hops

Cultivation of hops dates back to 200BC with the first record of being used in 1079 in Germany (Priest, 1996). This is a climbing plant that grows in a bine with a growing rate of 150mm (Priest, 1996) per day. The fruit is what is used in brewing. The scientific name *Humulus lupulus.* Its named after the glands lupulin glands which are sticky globes of essential oils and resins that are a main source of aroma and bittering compounds according to Priest in his 1996 book. Hops contain bacteriostatic properties which protect beer against biological spoilage microorganism. Hops, as described, inhibits the majority of Gramme positive bacteria while leaving gram negative bacteria alone. Following harvest, whole hops are dried (Bamforth, 2009).

2.2.5 Sugar

Sugar or what is called "adjunct" by the brewery the research was carried out from is a supplement of sugar for the extra extract that the yeast will use to produce more alcohol. These are either added in the adjunct cooker or during the wort boiling process. The main issue in brewing is the transfer of bacterial spores, primarily from *Bacillus spp*, which can withstand heat treatment, including boiling, and may persist in the finished beer (Bamforth, 2009).

2.3 Primary Contaminants

There is very little that is published on the sources of contamination in breweries reason this research is being carried out. (Hornsey, 2004) was able to show that wort contains high levels of carbohydrates, proteins, nitrogen, initial oxygen, vitamins and mineral ions which are suitable for the growth of many microorganism. Over the years, many changes have occurred with the idea of being able to use the same yeast strain in the production of beer to prevent contamination.. Hornsey also discovered that during the largering process, contamination is inevitable and the control is one way in which to prevent primary microbial contamination. In his book, he also suggested CIPs of the yeast lines as a way the brewery can handle its hygiene. In Primary contamination, contamination occurs through the brewing process through leaks, through sampling which is not aseptic and most importantly through raw material handling. For primary contaminations, wort could also get contaminated in the lines ass its being transferred from one vessel to another, through dead legs and also worn out floor surfaces (Back, 2009). A major contamination point in the brewery during the research was during the cooling of hot wort which uses a heat exchanger that was leaking and had mold and slime around it.

The raw materials should be kept in a clean and dry area with the usage of sterile water during the milling process. The use of sterile water is recommended for effective CIP, and the mashing temperature should begin at 50°C. This eliminates Lactobacillus and Pediococcus species (Briggs, 2004). These are likely to be present until the filtration process is completed.

As a brew is cooling, the prevention of most micro-organisms is through effective cleaning and sanitizing. Sterilizing is the system that's also used by different breweries around the country. This prevents micro-organisms like *lactobacillus*, *Pediococcus* and *Enterobacteriaceae* (Rainbow, 1978). As the brew is being cooled, anaerobic bacteria maybe observed. From the research paper written by Tendai on opaque beer, *Pectinatus* and *Megasphaera* were observed in his research and this is a clear indictation that anaerobic bacteria can be observed. One recommendation from the research was to have effective CIPs meaning the pressure, flowrate, detergent concentration and temperature must be aligned with the standardized requirements of the brewery. Effective CIP of the heat exchanger, use of either sterile oxygen or air and also aseptic yeast handling especially during yeast pitching.

Moving to the next part of the brewing process that has a high potential to cause of primary contaminants, *O.proteus* and a few other facultative anaerobes can be observed (Quain, 2002). *Lactobacillus and Pediococcus* can be picked during this process because of the yeast addition into the wort. Yeast addition is done after the addition of sterile oxygen or air is added in the wort. It is at this point where breweries have frequent CIPs before pitching, after pitching and before collecting the wort. Also, CIPs of the hoses, yeast lines and acid CIPs of yeast storage vessels is highly recommended as observed in the brewery the research is being carried out.

Aerobes such as *Micrococcus*, *Acetobacter*, and *Gluconobacter* were found during filtration (Bamforth, 2009). *Lactobacillu*s and *Pedicoccus* are facultative anaerobes as mentioned earlier. Beer is likely to be contaminated by anerobic bacteria such as *Pectinatus* and *Megasphaera* spp (Lounatmaa, 1987). CIP of all filtration vessels and hoses on a regular and effective basis, use of sterile vessels, sealing of all leaks, and use of sterile dilution water are all methods of prevention. Oxygen is said to be bad for beer because it oxidizes the Sulphur in the beer thereby giving a very bad off-flavor which is said to be rotten egg or sulphur oxide there by reducing the shelf life of the beer.

2.4 Secondary Contaminants

Secondary contaminations occurs where tunnel pasteurization is not being done. This occurs during bottling, canning and/or kegging. Those that do not use tunnel pasteurization responsible for at least half of the incidents of microbiological spoilage in breweries (Back, 1997). As a result, any point that comes into direct or indirect contact with cleaned or unsealed bottles is a potential source of contamination. Secondary contamination is most commonly caused by the sealer (35%), the filler (25%), the bottle inspector (10%), the bottle washer due to dripping water (10%), and the environment near the filler and sealer (10%) (Back, 1997).

According to Back (1994), microbial contamination in the filling area are a substantial consequence of initial growth of micro-organisms. *Acetic acid bacteria* and traces *Enterobacteria* grow in corners, dead legs or unclean areas where traces of beer or other products settle. Interesting part is that they are not considered harmful to the consumer or the beer but the fact that they are mainly slime producing bacteria, they tend to protect accompanying microorganisms from death through drying or disinfection. If these are present for a long time, the yeasts start growing in symbiosis with the *Acetic acid bacteria.* Yeasts produces growth factors that help lactic acid bacteria grow. Beer spoilage organisms such as *Pectinatus spp*. that can then metabolize the lactic acid produced by the latter organisms to propionic acid (Briggs, 2004).

All of the bacteria and wild yeast found during filtration are likely to be carried over to packaging as earlier discussed. Secondary contamination is avoided by thoroughly cleaning and rinsing returned bottles and kegs. Other preventative measures include using the proper detergent, being cautious, and standing time is critical. Assuring that oxygen levels, even in packaged beer, are below the company's specifications. As research has proven, aerobes grow rapidly when oxygen levels are high. In an article written by Dr. Ing entitled beer spoiling micro-organisms- an overview insists that brewing technologists and laboratory managers can quickly obtain technologically relevant information about the types of beer-spoiling microorganisms. Positive detection of the newly identified obligate and potential beer spoiling microorganisms presents a challenge for the field of brewing microbiology.

As a result, modifying established nutrient solutions may be required to some extent, especially if microorganisms only grow in particular products with lowered selective parameters ((Storgårds, 2000). A few articles have been published about microbial contamination in breweries with emphasis on the

categories depending on their spoilage characteristics. According to (Coint, 2022) in her article about beer spoilage microorganisms in a large scale and small scale market. She identified a number of microorganisms that can be used in this discussion. Opaque beer is also susceptible to these microorganisms but the fact that fermentation occurs in the already packaged bottle, that could be a reason why there are a few cases of microbial contamination in opaque beer.

2.4.1 Lactic acid bacteria (LAB)

Lactic acid bacteria (LAB) are the most common beer spoilers, accounting for 60-90% of microbiological incidents (Back, 2009). Lactic acid bacteria are widely regarded as useful microorganisms in the food industry and are found in a variety of fermented foods. However, it can be harmful and cause spoilage in many consumables (Suzuki, 2011).

2.4.2 Lactobacilli

Lactobacilli are the most common beer-spoilage bacteria, regardless of beer type (Thelen et al, 2006). Lactobacillus is the largest genus among the lactic acid bacteria and includes many species, but only some of them can deteriorate the beer (Jakobsen, 1996). However, there many different species that vary in their ability to grow in a brewery and also be able to withstand the anti-bacterial properties of hops.

2.4.3 Lactobacillus brevis

Lactobacillus brevis is the regarded as the most common bacteria found in beer because it is the worst beer spoilage organism in a brewery with record of about 50% of cases (Vaughan, 2005). This is an obligate hetero-fermentative bacteria which is tolerant to hops and grows at a pH of 4-5 and a temperature of 30°C (Campbell, 1996). *L. brevis* behaves versatile and could cause attenuation in beer due to his ability to produce haze and cause acidification through the fermentation of starch and dextrins (Back, 2005). There exists a few articles about the observation of

2.4.4 Pediococcus

Pediococci species are spherical shaped lactic acid bacteria. These have a fermentative physiology and grow best at low pH of about 4-5 and in the absence of air (Briggs, 2004). This species is associated with various food fermentation and the most dominant one is in beer production mainly because of their resistance to hops alpha acids. Unfortunately, their presence in a brewery may lead to the most feared off flavor of breweries which is diacetyl/acetoin formation, resulting in a buttery taste (Ouwehand, 2004).

2.4.5 Acetic acid bacteria

This bacteria oxidizes ethanol to forma acetic acid, hence the name. This is used in the production of vinegar. This is a gram negative bacteria that has few genera with the most common being *Acetobacter, gluconobacter and Gluconoacetobacter.* This was studied by the pasteurization forefathers Pasteur, Hansen, Henneberg and Beijernck (Rainbow, 1978). The acetic acid bacteria are Gram negative,

catalase-positive, oxidase negative, non-sporing, motile or non-motile short or coccoid rods, exhibiting strictly aerobic metabolism (Back, 2005). They generate energy through the incomplete oxidation of sugars, alcohols or other carbon compounds producing acetic acid as a byproduct. Over oxidation results in the production of water and carbondioxide (Campbell, 1996).

2.4.6 *Enterobacteriacea*

These are gram negative facultative aerobic bacteria that can grow in the presence /absence of oxyegen and highly inhibited by any alcohol and low pH. These are responsible for the spoilage of beer in low alcohol beers. They are catalase positive and oxidase negative (Back, 2009). In breweries, they are non motile and are rarely found in wort environments and in beer, they are non-pathogenic (Campbell, 1996). They are capable of producing off-flavours in through high levels of contamination of culture yeast at 8°C by forming acetoine, dimethylsulphide (DMS), and dimethyldisulphide ("celery-taste"). DMS is also found in malt and is expelled through wort boiling. Other evidence of DMS in the product is an indication of contamination by *Enterobacteriacea.* In addition, according to the data in Priest's Brewing Microbiology book, which is where all of this is cited, these bacteria secrete peptidases and proteinases into the substrate, which may reduce the beer's foam stability at high contamination levels.

2.4.7 Obligate anaerobic bacteria

At times, mixed populations of *lactobacilli, Pediococci*, *Pectinatus*, and *Megasphaera* contaminate beer. In these situations, spoilage typically occurs in two stages: first, lactic acid bacteria emerge and scavenge leftover oxygen to produce lactic acid, which is then used as the main carbon source by the strictly anaerobic beer spoilers, *Pectinatus* and *Megasphaera* (Back, 2005)

2.4.8 Pectinatus spp.

Pectinatus species are gram negative bacteria that are strictly anaerobic, spherical and is a common contaminant of non-pasteurized beer (Chelack, 1987). It was recorded that its first appearance and identification was by S.Y Lee in a brewery in Japan in 1978 with isolates from the beer which were assigned to this genus. These have been identified in breweries in Japan, Germany and Scandinavia (Haikara, 1984). Traces of these are related to off-flavour known as rotten eggs which is a sign of microbial contamination too. These species are sensitive to alcohol and are domiant in low alcohol beers.

2.4.9 Megasphaera cerevisiae

Megasphaera, like *Pectinatus* was described in 2006 is by far less common than *pectinatus* as beer contaminants. It is responsible for minor contaminations in breweries according to (Haikara, 1984) with a percentage occurrence of 3 to 7% of bacterial beer incidents. *M cerevisiae* a gram-negative, strictly anaerobic, and extremely harmful beer spoiling organism. Its formation of oval or round cells, usually in pairs or chains of four, sets it apart from *Pectinatus. M. cerevisiae* causes strong off-odors and off-tastes in beer, but it only causes very slight hazes and nearly undetectable sediments (Haikara, 1984).

Numerous organic and fatty acids, such as butyric acid and trace amounts of acetic, isovaleric, and valeric acid, are produced by *Megasphaera* strains. Additionally, sulphur dioxide (H2S) is generated (Lounatmaa, 1987).

2.4.10 Wild Yeast

Wild yeasts are any strains of yeast during the production of beer other than pitching yeast. These are gotten mostly from the environment of from the brewery surrounding (Gilliland, 1967). In most cases, they are inoculated intentionally in the production of Lambic beer and unintentionally through contamination in lagers, opaque beer, wines and whiskeys. The definition of wild yeasts is ambiguous, but it is traditionally divided into Saccharomyces and non-Saccharomyces (Quain, 2001). This definition covers non-brewing yeasts that got into the brewery through the air or raw materials, as well as brewing strains used for a different style of beer that may have been cross-contaminated. Despite wort boiling to sterilize it, it is cooled and later inoculated with pitching yeast but through unclean pitching lines of dirty yeast storage containers, a lot of unwanted yeasts can. These were reported to be found in atleast 41% of pitching yeast (Jerpersen and Van, 1998). Contamination of beer through wild yeast, a phenolic off flavour is an indicator. Some strains are unable to utilize dextrins which are present in beer to contribute to the mouth feel and body of the beer. In the production of opaque beer, the pitching of yeast is where microbial contamination occurs. In an article written in 1986, Novellie stated that the gelatinized malted sorghum are cooked and cooled and yeast is later added in the process. The yeast is inoculated at ambient temperatures and this attracts microbes ((Novellie, 1986).

2.5 Pasteurization

As stated earlier, pasteurization is a gentle heat treatment of beer to reduce the microbial load, i.e. the number of micro-organisms is reduced with a minimum of change in the aroma and flavour of the product. Other than CIPS as a way of preventing the consumer from harmful microbes in the beer, the last stage of microbial control is pasteurization. In most breweries around the world, different methods of pasteurization are used. Higher pasteurization temperatures have been found to make up for hygienic regulations. Inadequate sanitation will harm the beer before it reaches the pasteurization point. Thus, the best way to solve the issue is to follow general good manufacturing practices and raise the standard of hygiene rather than raising the pasteurization temperature.

In wineries, pasteurization—a procedure used to preserve wine to extend its shelf life—was initially introduced between 1865 and 1867 (Loeffler, 2006). The pasteurisation principle is attributed to Louis Pasteur, a French scientist who received a patent for the heat treatment of wine in 1865. But Swedish scientist Carl Wilhelm Scheele experimented with heating goods to extend their shelf life in 1782 (Dagan, 2006). Even though the majority of these early experiments' results were forgotten, Pasteur eventually discovered and developed them.

The primary goal of pasteurising beer is to increase the finished product's biological stability. The pasteurisation equipment requirements from the user's perspective must be met in order to produce

results that are acceptable. This implies that, for example, the pasteurisers should be simple to use and maintain, and their construction should be engineered to prevent the spread of infections. Other ingredients (such as sugars, peptides, amino acids, and oxygen) in the bright beer may react during heat treatment, affecting the final product's quality. Furthermore, there will always be a certain quantity of microorganisms in the bright beer that must be eliminated during pasteurisation which is emphasised (Sharpton, 2006). Even though factors other than pasteurisation efficiency determine the specifications of beer, it's important to remember that some of these factors—like high alcohol content, high degree of fermentation, high carbon dioxide content, high hop constituent concentrations, and low pH—will all have a highly beneficial effect on the outcome of pasteurisation (Dammann, 2011). The pasteurization of opaque beer is done through exposure to heat for the shortest possible time before the pitching of yeast. An article written with emphasis on opaque beer pasteurization stated that the beer that was pasteurized for 240 seconds developed off-flavours and was not favoured by the tasting team ((Nyamunda, 2017)

The most crucial requirement for a pasteurizer's performance is that it must be able to achieve the following fundamental goals: a minimum amount of sensory changes in the beer and adequate inactivation of microorganisms and enzymes through heat treatment. The type of pasteurisation that is currently used in breweries has changed. 21st-century breweries employ flash pasteurisation and tunnelling. Both benefits and drawbacks come with these.

There are several methods of removing or destroying micro-organisms in the brewing industry. The most common are:

- In-Package Pasteurisation
- Flash Pasteurisation
- Sterile Filtration coupled with sterile filling (not the major interest)

The specification for this type of pasteurisation normally states that a controlled application of sufficient heat to the product is necessary to ensure that all beer-spoiling micro-organisms are permanently inactivated while minimising product damage. The process should be maintained during machine and line stops and during start-up and run-out conditions. The smallest amount of water possible should be used as the corrective medium. Energy, also, should be conserved.

The second method, flash pasteurisation, pasteurises the beer in bulk before it reaches the filler. Here, much higher temperatures are used, but for a much shorter period of time.

2.5.1 Tunnel Pasteurisation

The majority of breweries in Zambia use this. This entails moving the beer cans or bottles through a tunnel with varying temperatures for a predetermined period of time. A conveyor belt moves the sealed and filled bottles or cans through the tunnel (Calina C, 2022). To adjust the heating and cooling, the containers are sprayed with water at different temperatures as they go through the tunnel.

A tunnel pasteurizer is characterised by its division into several zones, each with a different operating temperature. Thus, after the incoming containers are gradually heated to the proper holding temperature, the majority of the heat treatment is applied to them (Janousek, 2001). In the same way, the containers can be cooled down from the holding temperature to the required release temperature step-by-step. Beer is normally kept in its holding section at 60ºC. If more PU is required, it is typically achieved by increasing the treatment temperature rather than extending the machine's transit time (Dammann, 2011).

Tunnel pasteurisers can be configured as machines with one, two, or three decks. For a given floor area, the latter two configurations—two or three levels of containers within the same enclosure and sharing the same water system—offer a higher capacity (Dagan, 2006). A water distribution system, a circulation pump, and a base-functioning water tank make up each tunnel segment. Spray water can be applied to the can bottles in two different ways. Spray pans or spray nozzles can be used.Spray pans have a perforated base that increases the machine's width while distributing water evenly over the transport deck or decks. Alternatively, the water can be distributed using pipes equipped with spray nozzles. To ensure that every container has equal contact with the spray water, the nozzles are designed to produce an overlapping spray pattern. Generally speaking, large bore nozzles are used to minimise blockage. Pasteurizers that use spray nozzles may have various spray systems for each deck. The main consideration in the design of every tunnel pasteuriser is ease of access for cleaning. To make it simple to physically remove scale, broken glass, and other debris, large access doors should be installed on the tanks, water distributors, and strainer boxes. Spray nozzles should be easy to remove for upkeep and to replace when necessary. Additionally, spray pans need to be easily accessible for cleaning and inspection. All this is in refence to the article written by Dammann with regards to brewing science in his 2011 article.

The process of gradually heating and cooling the receptacles lessens the chance of thermal shock, which could shatter glass bottles which was experienced in the studied brewery when over pasteurization was done. In general, temperature differentials of between 22°C and 25°C during heating and between 18°C and 22°C during cooling are ideal for most glass bottles ((Dagan, 2006). This was observed in his article where he states that tunnel pasteurization is not sufficient for opaque beer. The pressure that is created inside the bottle during the pasteurisation process is another factor that must be considered. The pressure above the liquid, which is also referred to as the "headspace," is established by the gas volume (Janousek, 2001). For the different brands that run on the packaging line to the pasteurizers, they had a target percentage of the CO_2 that is dozed in. Generally speaking, the "headspace" should not be less than 3% of the bottle's total capacity (Back, 1997) that provides evidence of the connection between this and the pressure generated during pasteurisation. A control loop for each zone is used to regulate the temperature in a tunnel pasteurizer. Also in Tendai’s research, he discovered that the conditions in the packaging hall were significantly affected by the large surface area. He demonstrated that this must

be considered when designing the ventilation and heating systems. It is possible to insulate the warm zones of the pasteurizer, which reduces the heat transfer, even though it is typically not necessary for beer pasteurisers. There's a chance that a lot of water vapour will be produced because the pasteurizer operates using hot water sprays. Because of the high humidity, this water vapour can escape into the surrounding air and cause problems. To address this, most pasteurisers have an extraction system that releases the vapour into the atmosphere.

The most dependable and efficient method for guaranteeing microbiological stability and product quality is probably in-package tunnel pasteurisation is observed in the research dissertation by (Storgards, 2000). Because it takes place after filling and closing, it gets rid of both the naturally occurring microorganisms in beer and those that are added during those processes. No matter how much control is used, heating has a detrimental effect on beer flavour and this is one of the findings in this research. By maintaining the lowest possible pasteurizer times and temperatures, this effect can be minimised. To survive the high internal pressures that cause the container to build up during pasteurisation, stronger, more resilient packaging is required. As a result, production costs increase. Energy and water consumption result in high operating costs even with the use of sophisticated heat regeneration and water reclamation systems. The machine and installation come at a high cost. A complex device requiring maintenance, the tunnel pasteurizer has numerous conveyors, drives, pumps, and control instruments. Large floor area is also required. This is thought to be a significant drawback of tunnel pasteurisation.

2.5.1.1 Process Hygiene, Cleaning and Disinfection of Tunnel Pasteurizer

The fact that the product is heat treated while it is still sealed in its final packaging is one of the key advantages of using a tunnel pasteurizer. Since no machine part comes into direct contact with the product, the risk of contamination is negligible. However, the combination of heat, moisture, and nutrients from any beer leaking from defective containers could cause a tunnel pasteurizer to become a source of airborne infection, which could contaminate other parts of the packaging machinery. Odours that are disagreeable can also arise from algae growth caused by poor cleaning throughout the packaging facility. The majority of these problems can be easily fixed by routinely emptying the water tanks and flushing with clean water. The state of operation of the machine determines the frequency. Weekly tank draining is usually convenient, but it is possible to extend this to two or three weeks.

From this research, we can conclude that the major advantage of using tunnel pasteurisation is that it destroys micro-organisms naturally present in beer but also destroys micro-organisms introduced during filling and sealing. And the major disadvantages are heating has effect on beer flavour, it is kore expensive because the bottles that are used are those that are resistant to breakages due to the high temperatures of water and high internal pressures. It has a high operating cost which is electricity and water, installation of the equipment is expensive. Because of this fact, opaque beer doesn’t not go

through tunnel pasteurization because the beer still needs to have yeast before packaging. After its pasteurized, it is cooled to a temperature between 18°C-23°C to give chance and room for the yeast to grow and ferment the beer (Nyamunda, 2017)

2.5.2 Flash Pasteurisation

Also known as plate pasteurisation. Here, beer is raised from 2-4℃ to 70℃ for 20-30 seconds and then rapidly cooled back to process temperature. Depending on the flow rate, total residence time is 120 seconds (Dirksen, 2005).

Typically the shelf life of products emanating from the two processes are distinctly different. Usually home sale large pack (Keg) products have a shelf life of 6-8 weeks whilst small pack (Cans and bottles) have a shelf life of 36-52 weeks. Arguably, such differences can be related to the degree of microbiological risk associated with tunnel and plate pasteurisation which in turn, are bench marked against sterile filtration.

Directly from the light beer cellar, filtered beer with a temperature of about 1°C is pumped to the flash pasteurizer's heat exchange unit. Here, hot water circulating in nearby pipes rapidly heats it to 71°C, the pasteurising temperature. Only for opaque beer that's not filtered, it still applies because it is filtered in bulk before it is packaged (Novellie, 1986). After exiting the heat exchanger, the beer is transferred to a holding plate and kept there for roughly 20 seconds at the pasteurising temperature. After that, the beer is pumped back to the heat exchanger and goes through a number of piped sections that are intended to cool it down to around 2°C (Nyamunda, 2017). The beer then either goes straight to the filler or to a sterile holding tank. Controlling the pasteurisation temperature and duration is crucial, just like with in-package pasteurisation. The beer also needs to be kept under some pressure in order to maintain the desired level of carbonation. A buffer tank is necessary if flash pasteurisation is used in-line, and this could lead to infection issues if the tank isn't totally sterile (Back, 1997). Maintaining the sterility of the entire system, including the filler, is one of the most important prerequisites for flash pasteurisation (Calina C, 2022). Reusable bottles need to be thoroughly cleaned, and all flash pasteurisation equipment needs to follow a rigorous cleaning and sanitization regimen. To avoid reintroducing microorganisms, sterile filling and closing procedures should be carried out under strict control. It is necessary to routinely clean and sanitise the seamer or crowner.

When it comes to controllability, flash pasteurisation is a more straightforward process than in-package pasteurisation. The heat exchanger type that is employed produces lower discharge temperatures and uniform pasteurisation temperatures, not a cold area. Bright beer's low oxygen content and short pasteurisation period reduce the likelihood of oxidation and off-flavors (Storgards, 2000). Less maintenance is needed, the flash pasteurizer is relatively small in size, uses less energy, and has lower capital and operating expenses. Since the containers are not subjected to the high pressures created during in-package pasteurisation, lightweight and consequently less expensive packaging can be

utilised. These few points are considered to be the advantages of the flash pasteurisation. All these are said to be advantages for using flash pasteurization in a brewery.

The main drawback is that after flash pasteurisation, a fully sterile filling procedure is required. Microorganisms can unintentionally get into the beer through a variety of channels, including the filler, crowner, or sealer; packaging materials; or coming into contact with non-sterile pipes. Furthermore, microbes in the air can be captured by containers. Contamination can also come from in-line buffer tanks. In a few cases, labelling issues arise when containers are filled or damp with condensation. The use of stop-start fillers has a negative impact on the flash pasteurisation process because the beer needs to be redirected into a buffer tank to prevent high temperatures during the process's interruption. Sometimes the buffer tank is already full, which means that the beer may still be boiling during the flash pasteurisation process. Regular cleaning and maintenance are necessary to keep equipment from breaking down or operating inefficiently. If the beer is not regularly microbiologically checked, inadequate pasteurisation might happen.

2.5.3 Sterile Pasteurization

Sterile filtration is a totally different method of achieving biological stability in that it physically filters out the micro-organisms rather than destroying them through heat (Dagan, 2006). Used for beer in PET and draught containers, this method utilises a series of fine filters to remove micro-organisms. Briefly, beer from the bright beer cellar is pumped to a filter system and allowed to slowly flow through the filter, which traps the unwanted micro-organisms (Calina C, 2022). The beer then passes to the filler. Several different types of filters can be used, including diatomaceous earth, cellulose pulp and membrane screens. The last is the method used in most Namibian clear beer breweries, using very fine Millipore screens (Embashu, 2018).

When using the sterile filtration technique, microbes must not re-enter the beer during the subsequent filling operation. A sterile filling operation and clean bottles is thus a pre-requisite. In addition, sterile filtration is only effective if strict control is exercised over the microbiological population at all stages of the brewing process. The fewer micro-organisms that are in the bright beer as it enter the filter, the more effective will be the process (W, 2020).

A strict regime of cleansing and sanitation is important, and filters require regular flushing with hot water and sanitising. A benefit of using this type of pasteurization is the most obvious is it has no adverse effect on beer taste or smell, since no heat is used (Back, 1994). The beer is particularly clear after passing through the very fine filters. Also low capital and operating costs, low energy use, mechanical simplicity, requires little floor space, and containers need to be less robust and thus are less expensive. All these are reasons why sterile filtration is used instead of tunnel or flash pasteurization. Disadvantages of using this border around the requirement for absolute sterility in the filling operation which is said to be the most serious disadvantage (Dagan, 2006). Coupled with the fact that beer

supplied to the filters must be as free of micro-organisms as possible, a high degree of dedication by production staff is necessary to achieve this situation (Coint, 2022). The quality of the filters and the care and expertise necessary for packing and sanitising these filters is also important. Once the filtration process has begun, no adjustments or improvements can be made. Millipore screens are expensive and their life span is dependent on the number of micro-organisms in the beer. The filtered beer passes directly to the filler, and although samples can be taken at this stage and tested for micro-organisms, by the time the results are known the beer has been packaged and distributed to the trade (Calina C, 2022). Only short production runs are possible since a great deal of time is needed for flushing, re-packing and sanitising the filters. With the last reason, that's why macro breweries do not use this type of pasteurisation.

With all these methods in place to prevent contaminated beer on the market, Tendai published an article that discussed the effectiveness of flash and tunnel pasteurization at a local brewery in Zimbabwe. Based on his observations on opaque beer, there was an overall in the instability of beer properties which could have been due to various factors which include product contamination (Probably in the pipes due to improper pipe flushing or due to dirty filler heads), under-pasteurization of beer in the pasteuriser.

CHAPTER 3

3.1 MATERIALS AND METHODS

Materials used in this research include, agar plates, NBB-A agar, NBB-B Agar, NBB-C Agar and WLN Agar procured from Döhler GmbH (Darmstadt, Germany). Plate Count Agar, Pour Plate Agar, Copper Sulphate Agar and Yeast and Mould agar procured from ChemLab Supplies(South Africa). Spread plates, petri-dishes and sterile sampling kits procured from local suppliers in Lusaka. For the biochemical tests, 5% Hydrogen peroxide, Pottassium Oxide and 1% Aqueous solution of N,N,N,N-tetramethyl-p-phenylenediamine.

3.2 Study Design

This was a cross sectional study in which samples were taken during the different stages of beer production. Samples were collected randomly but at specific stages on random days.

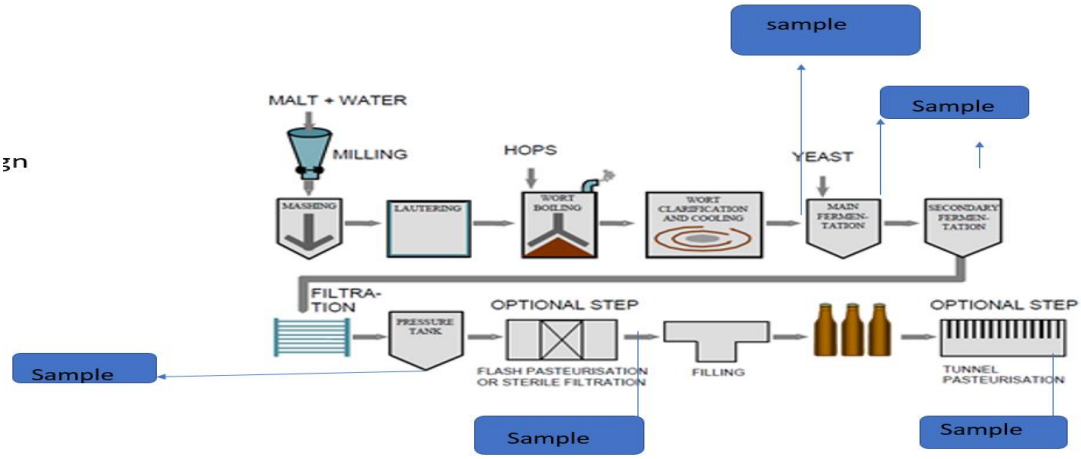


Fig 3.1: Image showing a summary Brewing Process (Storgards, 2000) and all points at which samples for analysis were collected.

3.3 Sample size

The sampling method that was used in this research is the purposive sampling. In this technique, the fact that it's a non-probability sampling method that was 100% based on my judgement but with a number of supportive factors to aid in judgement. The category used was the heterogenous or what is called the maximum variation purposive sampling because the selection of samples was done based on different characteristics like temperature, yeast availability, oxygen, carbon dioxide and the different heat treatments. In this particular case, for every beer batch that was exposed to oxygen, a sample was taken. When inoculated with yeast at a certain temperature, a sample was taken, when yeast was removed and temperature reduced, a sample was taken. When filtered and carbon dioxide introduced,

a sample was taken and after the two different heat treatments that were available. For every beer batch, 6 samples were collected.

3.4 Sample Collection

Four different tanks were analysed from the milling stage till the beer was packaged that was selected randomly for this research. The total number of samples collected were 24 samples. Each beer batch with names labelled Batch 1, Batch 2, Batch 3 and Batch 4 had 6 other samples that gave a total number of 24 samples. Please take note that these were the code names given to the samples to avoid mentioning the actual names and actual brands from the named brewery. This was done weekly for a period of 3 months to allow proper and correct data collection and analysis. These samples were collected on different days, at different times firstly whilst they were in sugar state at different stages of beer production which were stated earlier. Also, after they were filtered, samples were taken at BBT stage before pasteurization for analysis in the lab under aseptic techniques. Samples after the filler which is before pasteurization and before the labeller which is after pasteurization were analysed in a lab under aseptic techniques.

3.4.1 Wort Samples

Wort samples were collected whilst the brew was being cooled just before pitching. In a tank where 2 brews are collected, only 500mls of wort sample was collected aseptically. Spraying the sampling point with alcohol and allowing it to dry to ensure that all microbes that may be present are eliminated.

3.4.2 Ferment Samples

In this stage the samples were collected when the wort has just started fermenting whilst still in the ferment vessel. These were collected aseptically by spraying the sampling point an allowing it to dry out. At this particular stage, the wort which was now being called green beer was allowed to drain for 30 seconds and the sample was collected.

3.4.3 Storage samples

At this point, the beer had already matured and was already being cooled to prepare for filtration. At this stage, the sample was collected aseptically by spraying with 99% ethanol and allowed to dry. The beer was then stored at 4°Celcius ready for inoculation on the agar plates.

3.4.4 Bright Beer Tanks

The beer has already matured and is already filtered. At this point, the beer has already been diluted and targeted at the right Alcohol. The sampling point was sprayed with 99% ethanol and was allowed to dry. The sampling point was left open and beer was allowed to run for 30 seconds to allow for clear beer that has no contaminants.

3.4.5 Pasteurized Bottles

At this point, the beer has been pasteurized and for tunnel pasteurization, they have been pasteurized and immediately they were out of the tunnel, the bottles were collected. Wearing gloves to ensure the

safety of the person collecting the samples. These were collected when they have already been packaged and are ready for distribution. This was the same case for the beer that was pasteurized using flash pasteurization.

3.5 Microbial Analysis

For the different types of micro-organisms that are associated with beer, there are specific agar media that are used. This is what was advised by the brewery and during the microbial analysis done as their routines. Different agar agar media were used. Because my interest was also yeast, a specific agar was used for the identification of wild yeast and culture yeast.

3.5.1 NBB- A Agar

This was a ready to use agar medium that is used to determine anaerobic bacteria in beer samples. These as mentioned in the literature review are beer spoilage microorganisms in a brewery. Because of the selectivity of the NBB media, the harmless accompanying flora such as culture yeast are inhibited. NBB- A agar plates were prepared and by pouring on the spread plates. Contents in the SSSB bottles were mixed homogenously through shaking. Using a pipette, a volume of 10 m/l was collected and poured on the plate. Using a sterile rod that was passed through a flame and cooled, it was evenly distributed around the plate. After this was done, the lid was put back on the plate, labelled and incubated for 5 days at 28°C.

3.5.2 NBB-B Agar

This is used for the determination of spoilage bacteria. This is performed in a CO_2 atmosphere in order to detect facultative or strictly anaerobic bacteria. NBB- B agar plates were prepared and by pouring on the spread plates. Contents in the SSSB bottles were mixed homogenously through shaking. Using a pipette, a volume of 1m/l was collected and poured into a sterile test tube with NBB-B agar. The anaerobic conditions were achieved through a gas pack system in an anaerobic jar and incubated for 5 days at 28°C.

3.5.3 NBB-C Agar

This is an agar medium that is always used for green beer, which is unfiltered beer. Properties of this medium is that it has a high selectivity in detecting micro-organisms. This is done through mixing a beer sample and a variable amount of sterile water with a pH of less than 5.0. Using a pipette, collect 10mls of NBB-C to a 180mls SSSB bottle containing 170mls of the sample. The bottle was mixed thoroughly to ensure the pH is less than 5. For the wort sample, it was incubated for 5 days and for the green beer, it was inocubated for 7 days at 28°C.

3.5.4 PCA Agar

This was used in the detection of aerobic bacteria in beer samples. This agar medium was poured into a plate after being autoclaved because it is already made to use. Using a sterile pipette, 1ml of the sample was collected and the lid was lifted slowly at 45° angle and expelled into the plate. The plate was

covered and swirled to allow even distribution of the sample. This sample was then incubated at 35°C for 2 days.

3.5.5 Wallerstein Laboratories Nutrient Media (WLN)

WLN is used for the enumeration and cultivation of yeasts and bacteria in the microbiological control carried out in the brewing and other fermentation industries especially of yeast. WLN has excellent differential properties, being particularly useful in distinguishing between culture yeast and Wild yeast. 10mls of the sample was collected aseptically and put in an SSSB bottle. For this particular agar, any volume is used. I collected 50mls of the agar and collected 10 mls of the sample and was put in a hot water bath that was at 95°C and allowed to melt. The bottle was gently swirled to allow proper mixing of the sample and the WLN agar. The contents of the melted WLN agar and the sample were poured using a pipette. A volume of 20mls was pipetted and transferred to a petri dish where it was allowed to solidify and incubated at 28°C for 4 days. This concept was also used with the Wort agar. The method used was the same for wort agar.

3.5.6 MYGP-Copper sulphate agar (Copper Sulphate Agar)

Copper resistance, coded by the *CUP1* gene, allows for the selection of industrial yeast strains from strains not resistant to copper. The copper concentration required needs to be determined for new production strains when introduced into a brewery. The agar was poured ona petri dish and 5 drops using a dropper was poured on the petri dish and using a flame sterilized rod, it was spread evenly on the agar. The petri dish was incubated at 28°C for 5 days.

3.5.7 Yeast and Mould (YM)

Medium is selective for yeast and moulds. Addition of copper to the medium will enable wild yeast to grown on the medium whilst supressing the growth of ale or lager yeast. The agar was poured ona petri dish and 5 drops using a dropper was poured on the petri dish and using a hot rod that has been cooled, it was spread evenly on the agar. The petri dish was inoculated at 28°C for 5 days.

3.6 Biochemical Tests

Biochemical tests done included catalase test, POH test, Oxidase test, Gram staining, motility and wet prep tests.

3.6.1 Wet Prep

This is done prior to any microscopic identification to see how the bacterial cells look. This was done by collecting a small portion of the colony using a sterile rod and put on the slide. A drop of distilled water was put on the slide and covered with a cover slip. included motility, It was observed under a

microscope. Observations included motility, what it used to move, shape and if they were observed in pairs.

3.6.2 Gram staining tests

A stain for the classification of bacteria into Gram-positive and Gram-negative types. The staining must be conducted on cultures not older than 48 hours. A heat fixed smear of bacteria was stained successively with a solution of crystal violet, and with a dilute solution of iodine. The preparation wass then treated with an organic solvent in this case alcohol, and then stained with Safranin. The cells of some bacteria known as Gram-positive bacteria, resisted decolourisation by the organic solvent, whereas the cells of Gram-negative bacteria were rapidly and completely decolourised. Gram-positive cells retain the deep purple colour conferred on them by the initial staining with crystal violet and iodine, whereas Gram-negative cells, which have been decolourised, exhibit the red colour of the counter stain. The mechanism of the Gram stain is based on consistent and major compositional differences between the walls of Gram-positive and Gram-negative bacteria.

3.6.3 Catalase test

This identification test involved using a drop of H_2O_2 5% (solution of catalase) to the colonies that have been fixed on a slide. If the colonies foam up, it means that they are catalase positive bacteria (aerobic bacteria) and if the colonies don't foam up, they are catalase negative bacteria (anaerobic bacteria). This test is used for the identification and differentiation of some microorganisms, for example, Lactobacillus from Bacillus and Pediococcus from Micrococcus.

3.6.4 Oxidase test

This method is used for the testing of cytochrome oxidase in microorganisms. Using the filter papers that are made up of the oxidase reagent, a drop of the bacteria was put on the filter paper and observed for any colour change.

3.6.5 Potassium Oxide (KOH) Test

It is a test to differentiate between Gram positive and Gram-negative bacteria by observing changes in the bacterial viscosity of the bacteria when reacting with KOH. A sterile loop was used to drop 3 drops of KOH solution on a microscope slide. The loop was re-sterilized by passing through a hot flame, allowed to cool and used to transfer from one isolated colony to the plate with the KOH. This was mixed well. Gel formation was expected for a positive test. And results were recorded.

3.6.6 Wild Yeast Identification

This is the combined inoculation method for wild yeast detections, respiratory deficiencies and yeast variants. Using a loop, a portion of the yeast colonies were put on a microscopic slide, a drop of distilled water was added and covered using a cover slip and observed under a microscope.

3.7 Bacterial Population

Serial dilution was used to reduce a dense culture of cells to a usable concentration level that allowed for the quantification of cell populations that were easier for this research. Serial dilution was done for samples that grew colonies after pasteurisation and that were between 30 and 300 on the plate agar.

To perform this serial dilution, several dilution blanks were first prepared. The samples that were serial diluted were KLSV02 tunnel and flash pasteurized, CBLSV21 flash pasteurized, KLLSV30 flash and tunnel pasteurized and MLSV08 tunnel pasteurized beer. 1ml of the original sample was put in a testube containing 9mls of distilled water, this was labelled as test tube 1. Test tube 1 was put on a vortex and 1ml was collected and put in another test tube labelled 2 containing 9mls of distilled water. Finally, 1ml was removed from test tube labelled 2 and transferred to test tube labelled 3 that had 9mls of distilled water. Plating of these was done according to the agar that was set for the type of bacteria that was supposed to be grown.

3.8 Secondary Data and source of literature

For the purpose of standardizing, analyzing and identifying the information gathered on the types of microorganisms associated with beer, secondary data was collected from a named brewery particularly for each identified microorganism using an identification tree cited in the references.

Fig 3.2

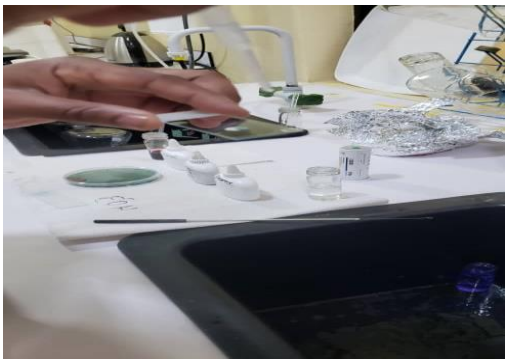


Fig 3.3

Fig 3.2-3.6 Sample preparation and isolation of microorganisms. Purification of bacterial and yeasts..

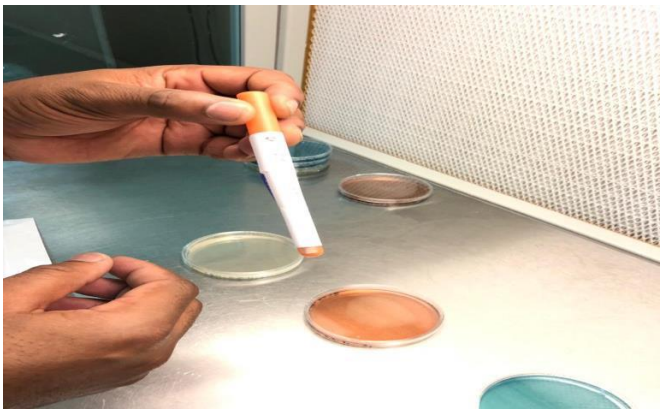


Fig 3.4

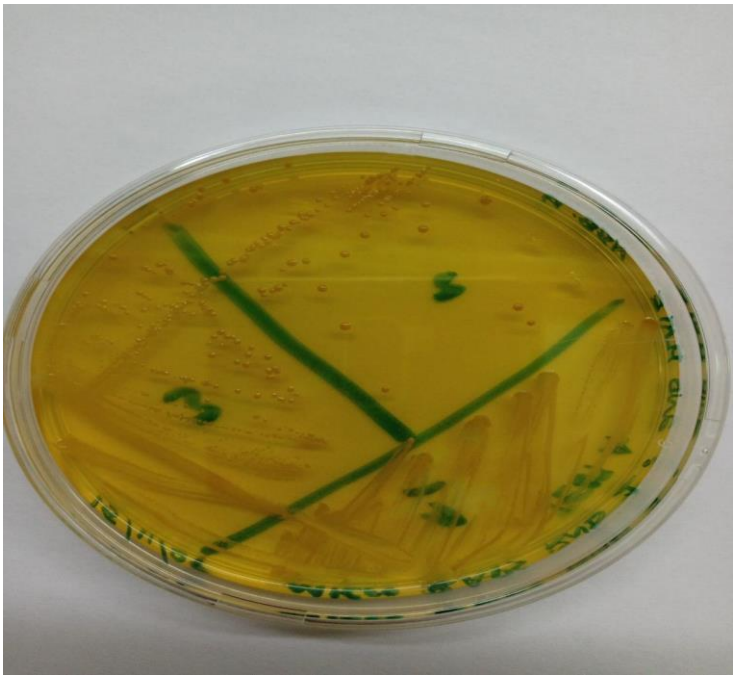


Fig 3.5

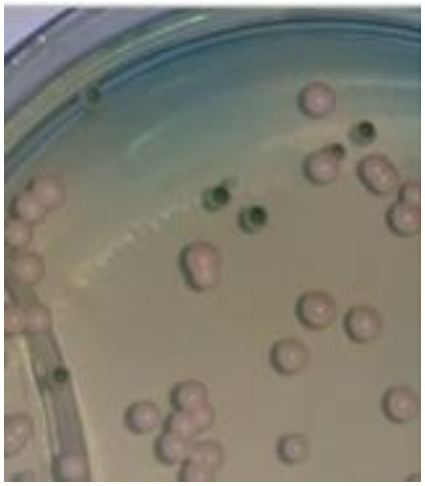


Fig 3.6 showing wild yeast colonies identified on a WLN agar.

CHAPTER 4

4.1 The presence or absence of microorganisms in the various beer samples.

Table 4.1 Results of examination of microorganisms in the various samples

Date		Batch 1	Batch 2	Batch 3	Batch 4
Wort	AB	Not present	Not present	Not present	Not present
	CY	Not present	Not present	Not present	Not present
FV	AB	Not present	Not present	Present	Present
	BS	Not present	Not present	Not present	Not present
	WY	Not present	Not present	Not present	Present
SV	AB	Present	Present	Not present	Present
	BS	Not present	Not present	Not present	Not present
	WY	Not present	Not present	Not present	Present
BBT	AB	Present	Present	Present	Present
	BS	Not present	Not present	Not present	Not present
	WY	Not present	Not present	Present	Not present
PB	AB	Present	Not present	Present	Present
	BS	Not present	Not present	Not present	Present
	WY	Not present	Not present	Not present	Not present
PK	AB	Present	Present	Present	Present
	BS	Present	Not present	Not present	Present
	WY	Not present	Not present	Not present	Not present

Key

AB: Aerobic Bacteria

BS: Beer Spoilage Bacteria

WY: Wild Yeast

SV: Storage Vessel

FV: Fermentation Vessel

PK: Pasteurized Keg

PB: Pasteurized Bottle

BBT: Bright Beer Tank

With specific reference to bacteria observed, the most common type of bacterial species were aerobic bacteria which had grown on PCA. Further tests gave the following results. The results in table 4.1 show that the most common type of microorganism in the brewing industry is Aerobic bacteria, followed by Beer Spoilers and a few traces of wild yeast which will be discussed later in the next chapter.

4.1.1 Batch 1 Samples

Table 4.2: Biochemical tests done on bacteria and observed bacteria

Organism	Biochemical Tests					
	Wet Prep	Catalase	Oxidase	Gram Stain	POH	Organism
SV	Bacilus, non motile	+	-	-	+	Acetobacter
BBT	Bacillus, nonmotile and did not exist in pairs	+	-	-	+	Gluconobacter
Tunnel Pasteurized	Bacillus, nonmotile and did not exist in pairs	+	-	-	+	Glucconobacter
Flash Pasteurized	Bacillus, non-motile and did not exist in pairs	-	-	+	-	Lactobacilus

4.1.2 Batch 2 Samples

Table 4.3: Biochemical tests done on bacteria and observed bacteria

Organism	Biochemical Tests					
	Wet Prep	Catalase	Oxidase	Gram Stain	POH	Organism
SV	Bacilus, motile	+	-	-	+	Zymomonas
BBT	Bacilus, motile	+	-	-	+	Zymomonas
Flash Pasteurized	Bacilus, motile	+	-	-	+	Zymomonas

4.1.3 Batch 3 Samples

Table 4.4: Biochemical tests done on bacteria and observed bacteria

Organism	Biochemical Tests					
	Wet Prep	Catalase	Oxidase	Gram Stain	POH	Organism
SV	Bacilus, motile	+	-	-	+	Zymomonas
BBT	Bacilus, motile	+	-	-	+	Zymomonas
Tunnel Pasteurized	Bacilus, motile	+	-	-	+	Zymomonas
Flash Pasteurized	Coccus, non motile and non spore forming	-	+	+	-	Pedicococcus

4.1.4 Batch 4 Samples

Table 4.5: Biochemical tests done on bacteria and observed bacteria

Organism	Biochemical Tests					
	Wet Prep	Catalase	Oxidase	Gram Stain	POH	Organism
FV	Non-motile	+	-	-	+	Gluconobacter
SV	Non-motile	+	-	-	+	Gluconobacter
BBT	Non-motile, spore forming	-	+	+	-	Pediococcus
Tunnel Pasteurized	Non-motile	+	-	-	+	Gluconobacter
Flash Pasteurization	Non-motile	+	-	-	+	Gluconobacter
Flash Pasteurization	Bacillus, motile, non-spore forming	-	-	-	+	Pectinatus

Table 4.6: Bacteria identified and frequency of appearance based on the different agar used.

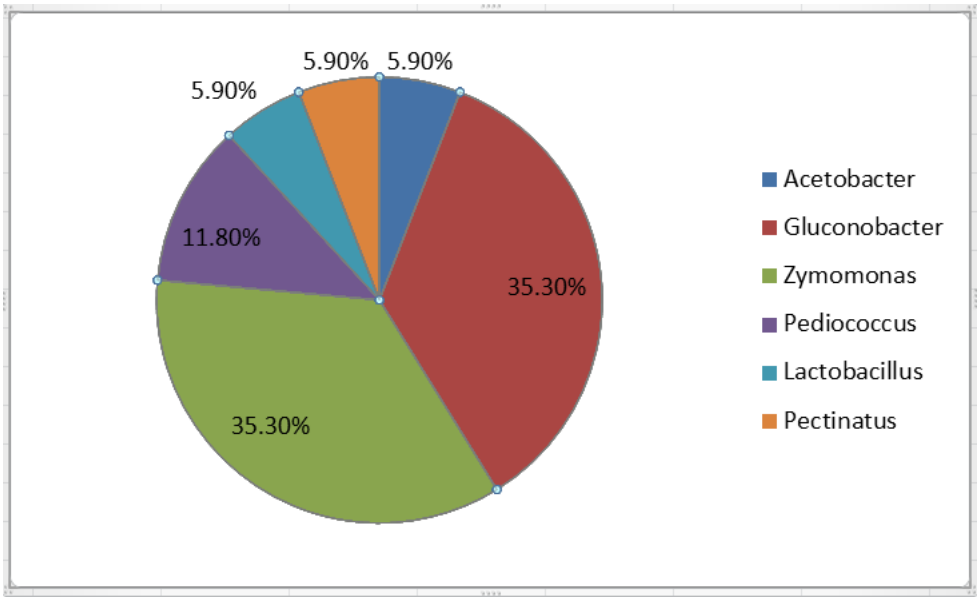
Aerobic Bacteria	Beer Spoilage
Acetobacter (1)	Lactobacillus(1)
Glucanobacter(6)	Pectinatus(1)
Zymomonas(6)	
Pediococcus (2)	

The identified bacteria have been summarised in the table inclusive the number of times they were identified in the different samples.

4.2 Bacterial Populations

Table 4.7 Population of bacteria in CFU/ml.

	SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4	
	Test tube 1	Test tube 2	Test tube 1	Test tube 2	Test tube 1	Test tube 2	Test tube 1	Test tube 2
BBT	$7.1X10^6$	$5.9X10^7$	$18.9X10^2$	$15.7X10^4$	$99.7X10^2$	$76.6X10^4$	TNTC	TNTC
TUNNEL PASTEURIZED	$4.7X10^5$	$38X10^3$	0	0	TNTC	TNTC	$29.8X10^5$	$11X10^7$
FLASH PASTEURIZED	$71X10^1$	$59X10^3$	$98X10^1$	$73X10^3$	TNTC	TNTC	$7.1X10^6$	$5.9X10^7$



Graph 1: Frequency in percentage of identified bacteria

CHAPTER 5

5.1 DISCUSSION

This research was specifically designed to evaluate the efficacy of the breweries' pasteurisation procedures. Samples from various brewing stages were collected in order to evaluate flash and tunnel pasteurisation. The results of the observation of microbial contamination areas indicate that yeast pitching is always a point of entry for microbial contamination. Microbial contamination is rare after the wort cools, unless thermophiles are found, which are caused by high temperatures during the breakdown of sugars into fermentable sugars. The temperature of the yeast is 11°C when it is pitched into the wort. Wort is heated to 14°C in the fermentation vessel, where fermentation takes place. Variously varied in properties, organisms were isolated and identified from various sections of the brewing process. The following microorganisms were found in this study: Lactobacillus, Pectinatus, Pediococcus, Zymomonas, Acetobacter, and Gluconobacter. The most frequently observed species was Gluconobacter. This is both prior to and following pasteurisation. A genus of bacteria belonging to the acetic acid bacteria is called Gluconobacter. As a spoiling organism, it was covered in the literature review. They cause rotting of fruits, such as pears and apples (Jakobsen, 1996).

Using samples that were examined both before and after pasteurisation, flash and tunnel pasteurisation were contrasted. After the microorganisms were identified, different results were obtained from the comparisons for each batch. After pasteurization micro-organisms observed were, Gluconobacter, lactobacillus, Zymomonas, Pediococcus and Pectinatus. According to literature, after pasteurization there should be no trace of any micro-organism present (Quain, 2001). The beer that was analysed in the laboratory and later tasted before being sent to the market as the standard procedure requires, had some tastes that affected the freshness of beer. In an article written on gram-negative bacteria in 2017, it states that the major off-flavour produced by the identified bacteria which are Gluconobacter, Zymomonas and Pectinatus is the rotten egg flavour that each of these beer samples showcased. This substance, known as hydrogen sulphide, has aerobic bacterial contamination traits. The species of acetic acid bacteria that were found did not deviate significantly from the theories that claimed they were always rotten egg flavoured. The sour taste in these samples was caused by the acetic acid bacteria (Gluconobacter) oxidising the ethanol, which produces vinegar (Ashtavinayak, 2017). When the beers were tasted, they revealed the qualities of beer tainted by aerobic bacteria as described in the review above.

5.1.1 BATCH 1

Batch 1 sample was the first batch to be collected at the beginning of this research. At wort and fermentation stage, there were no traces of any micro-organisms. As indicated in the Literature review by (Suzuki, 2011), wort is said to have a high tolerance for the presence of hop compounds and is able to survive under anaerobic conditions. A few microorganisms are said to metabolize sugars but end up producing lactic- acid instead of alcohol making the microorganisms that could be observed in this stage

beer spoilage organisms. Despite malt containing high levels of *lactobacillus* on the husks (Vaughan, 2005) that's insists that malt has high levels of the bacteria ,this brand showed no traces of any microorganisms despite it being a high malt brand. This could have been due to effective and frequent cleaning of the brewing and fermentation equipment. The alternative explanation is the possibility of using good quality hops which many microorganisms are sensitive too and adherence to boiling times and temperatures which was also mentioned in the literature review and has been researched.

At maturation stage numerous colonies of *Acetobacter* were observed. These were observed to be gram-negative bacteria which tested positive for catalase and negative for oxidase. These were not inhibited by hop compounds but they are said to grow rapidly in beer producing acid off-flavours like "acetic" (W, 2020).

After filtering the beer to prepare it for packaging, microbiological analysis was done again and traces of aerobic bacterial colonies were observed. The observed bacteria was acetic-acid bacteria which was classified and identified as *Gluconobacter*. These were positive for catalase and potassium oxide and negative for oxidase test and gram staining. After gram staining, cells were observed to be non-motile, bacillus and did not exist in pairs. This is said to be the most prominent representative of oxidative bacterial group that is able to carry out oxidative fermentation (Back, 2005). It is said to produce glucose to gluconic acid (Back, 2009) rather than ethanol to acetic acid , which differentiates them from other acetic acid bacteria.

After tunnel pasteurization, 65 colonies of POH and catalase positive colonies were observed. These results were those identified at BBT stage but with a few colonies of bacteria. These were *Gluconobacter spp.* In the flash pasteurized sample, a few colonies were observed. These were not *Gluconobacter* as initially identified in the BBT sample but these were identified as *lactobacillus.* This is a clear indication of improper CIPs of the lines and the beer containers. With these findings, a sample was tasted from this batch and this was said to be sour and acidic because of the acid produced. These were gram positive and were rod shaped. Oxidase and POH tests were negative.

Serial dilutions of this BBT, tunnel and flash pasteurized beer was done giving TNTC for the first 3 test tubes in the BBT beer. This is an indication that the number of cells still produced despite the reduction of the nutrient medium. In other samples, i.e. tunnel pasteurized gave a population of 47×10^5 cfu/ml in the first test tube being the highest number and 0cfu/ml for the last test tube. In the flash pasteurized beer, the highest was 39×10^6 cfu/ml being the highest in test tube 4.

With available literature on the effectiveness of flash and tunnel pasteurization, this sample after assessing proved that tunnel pasteurization is highly recommended due to the fact that the beer is exposed to high temperatures for a long period of time. The introduction of the new species of bacteria that was identified is in agreement with results from published studies that state that insufficient CIPs introduces microorganisms (Bamforth, 2009).

5.1.2 BATCH 2

In the wort and fermentation samples collected, there were no traces of any microorganisms. Reason could have been same reasons stated for KLSV02. Boiling time and temperatures could have been good and very good quality of the hops used. At maturation level which is also called storage level, several colonies were observed. These were bacillus and a few of them where motile. The positive tests observed were POH and catalase whilst the other biochemical tests were negative. The pickup in number of micro-organisms could have been a consequence of insufficient cleaning of the presence of dead legs in the line (Back, 2009).

At BBT stage, there was a reduction in the number of colonies that were observed. A total number of 210 colonies were observed and most showed signs of containing the enzyme catalase and tested positive for POH. Gram staining results showed to be negative and the oxidase test was also negative. The microorganism observed was *Zymomonas spp.* Because a few were motile and were bacillus and had no spores. These as mentioned earlier need glucose for survival. They don't survive in lagers (Briggs, 2004) and this could have been the reason for the reduction in maturation to BBT. Despite this, it is able to tolerate high alcohol levels.

The pasteurized beer had zero signs of any microorganisms. This is the reason why serial dilutions were not done for this sample. From these observations, it may be concluded that the was practically sterile and the tunnel pasteurization was 100% effective.

However, the flash pasteurized beer had traces of bacteria that grew on the plates. A total of 105 colonies were observed to have grown. This however was serially diluted and the highest population was 34 x 10^6 cfu/ml which was concluded to be the cell population of the stock culture . The identified sample was *Zymomonas spp* which showed a reduction from the sample without pasteurisation.

5.1.3 BATCH 3

At wort stage, no microorganisms were observed. At FV stage, many colonies of gram negative bacteria that was catalase positive, POH positive, oxidase negative, bacillus and a few motile cells were observed. Because of the tolerance and love of glucose, the beer tested positive for *Zymomonas spp*. It is capable of surviving in a oxygen dominated environment and an oxygen deficient environment. This means it is a facultative anaerobe. This is said to be a beer spoilage organism as well (Quain, 2002).

At maturation stage, probably because of the lack of glucose and high ethanol produced, there were no traces of any microorganism in this stage. CIP of the storage vessels and racking lines was efficient because that is the only chance microorganisms are groomed. Racking is the process of transferring beer from the fermentation vessel where fermentation takes place to the storage vessel were maturation of the beer and chilling of the beer takes place. If the racking line doesn't meet the criteria of CIP which are correct temperature, correct time, correct pressure and correct mechanical action, then the line is prone to any form of microbial contamination.

At BBT stage, the number increased to many colonies on the plate. These colonies resembled *Zymomonas spp.* These species were observed even after tunnel pasteurisation. After flash pasteurization, a different sample was observed. These were coccus and were identified as *Pediococcus.* This is a lactic acid bacteria as stated in the literature review. It is a gram positive, coccoid, non-motile and catalase negative bacteria. When tasted, it gave a buttery off-flavour which the brewery named as diacetyl. *Pediococcus* is said to be detected in late fermentations and also during the conditioning of beer which is also the packaging of beer. This could have been observed as 1 or 2 colonies on the plate but was unable to see its growth because of the numerous cells that were on the growth. It was however observed when it was serially diluted as a few colonies at BBT stage. At BBT stage, 16.9 x 108 was observed to the highest in test tube 5. After tunnel pasteurization, TNTC was observed in all test tubes that were serially diluted this means the concentration in the stock sample was highly concentrated. After flash pasteurization, the population results were TNTC in all test tubes. A clear indication was how the beer tasted. This beer was sour, acetic and contained a high buttery flavour which indicated microbial contamination because diacetyl is a sign of contamination. The production of this beer was done in a microorganism filled environment which allowed it to groom the growth of the observed bacteria. Starting from the fermentation vessel till it was packaged, the amount of microorganism that was present was too high for even the serial dilution to assist and allow the quantification of this sample. Pasteurisation which is the last defence that the beer had against any microorganism was not effective because the samples were not considered sterile.

5.1.4 BATCH 4

At wort, before fermentation, all samples were observed not to grow any colonies. *Gluconobacter* was observed because it was bacillus, non-motile, POH positive, catalase positive , oxidase negative and gram staining results were negative too. But at BBT state, *Pediococcus spp.* This may have been seen in maturation stage but colonies could have been too minimal to actually observe.

At BBT stage, bacterial population was dertermined and this gave 29 x 105 cfu/ml in the stock sample. The tunnel pasteurised sample showed traces of *Gluconobacter spp* which were POH positive, catalase negative, oxidase negative and is a beer spoilage bacteria. In this sample, all species were observed and different colonies were observed on all the plates that were inoculated. *Gluconobacter* as earlier mentioned gives beer an acetic/acid taste because of the acetic acid it produces (Back, 2005). He also states that it is found in dirty, empty bottles or containers returned from the market. It has a cell wall that has the ability to grow with a few common sugars found in plants and also in high concentrations of phenolics in or around the plant. *Pectinatus spp* was also observed as a beer spoilage in the identification of these bacteria. Although bacterial population was not determined for the flash pasteurized beer, these were identified as *Pectinatus spp.*

The introduction of the only micro-organisms allowed in the brewery *Saccharomyces* into the wort which contains highly fermentable sugars that produce alcohol and carbon-dioxide as stated in (Bamforth, 2009) which is highlighted in the literature review.

Observed trends for microbial diversity associated with beer production are less diverse than those in the environment. The cooled, oxygenated wort is pumped into a fermenter where Saccharomyces strains are added and sugars fermented to ethanol and carbon dioxide to rapidly convert the wort to beer as stated in Bamforth in 2002 which is highlighted in the literature review. The resulting conditions are hostile to the growth of most microorganisms.

Beer is high in ethanol and carbon dioxide, contains hop-derived antibacterial compounds, and is low in pH, oxygen, and residual nutrients, but about 20% of the reducing sugars in pure wort are oligosaccharides (Briggs, 2004). Many brewers are aware that it contributes to the mouthfeel and flavour of beer and promotes the potential for microbial dioxide spoilage (Bamforth, 2002). Due to the harsh conditions of beer fermentation, a unique group of yeasts and bacteria were selected that are specialized for growing beer. All species identified in this research are the most common contaminants of beer, from early fermentation to packaged products (Bamforth, 2002).

LAB spoils beer by acidification, haze formation and/or diacetyl formation, giving beer the intense flavour of artificial butter (Bamforth, 2002) reason why the KLLSV30 had a buttery taste. Pediococcus which is a lactic acid bacteria was observed in this sample. Many strains can also produce exopolysaccharides (EPS) in beer. This can lead to an oily consistency or, in extreme cases, to the formation of mucus, especially *Pediococcus spp* is known for the production of diacetyl and EPS and is a common contaminant in both lager and ale breweries due to its strong growth at low temperatures, following previous events at the brewery in 2019 problem since 2013 (Bamforth, 2002). However this was not observed for the 6 *Pediococcu*s colonies that were identified in this research.

Except for LAB, few Gram-positive bacteria in beer have been reported (Bamforth, 2002), which was reported in this research.

An important factor limiting which organisms can spoil beer (particularly ethanol-tolerant and pH-tolerant Gram-positive bacteria) is the presence of bitter compounds from hops (Bamforth, 2002). Hops contain many compounds that inhibit the growth of Gram-positive bacteria (Bamforth, 2002). These include, among others, isoalpha acids formed from hop alpha acids during the boiling of the wort (Bamforth, 2002). Isoalpha acids act as proton ionophores, dissipating transmembrane proton gradients, lowering cytosolic pH, and suppressing proton motive forces (Sakamoto and Konings, 2003), this was observed during at the analysis of FV samples that gave zero beer spoilage microorganisms at fermentation stage. This impairs enzymatic activity and nutrient transport, arrests growth, and ultimately leads to cell death (Back, 2005).

Aerobic Gram-negative acetic acid bacteria (AABs) pose a serious threat to beer production, but their activity in beer production can be neglected because exposure to oxygen can be avoided. In the past, when beer was barrel aged, without modern brewers having luxuries (e.g., conical steel fermenters and

controlled headspace), AAB was a more common threat, It is still commonly found in barrel-aged beers (Bamforth, 2002). Gram-negative bacteria identified as AAB were Acetobacter and Gluconobacter spp. These bacteria spoil beer by oxidizing ethanol to acetate, effectively turning beer into vinegar.

As the introduction of modern technology lowers the dissolved oxygen levels in beer, new threats replace old foes. These new contaminants were obligatory anaerobic *Veillonellaceae* organisms (Bamforth, 2002), including *Pectinatus* identified in this study. Members of this family belong to the Gram-positive phylum *Firmicutes*, but stain Gram-negative and have a lipid bilayer (Bamforth, 2002). Most veillonellae are found in aquatic sediments or in the gut of mammals, but the above have only been reported for beer, leading to haze formation, overwhelming production of propionic acid, acetic acid, hydrogen sulphide, mercaptans, and spoilage by inhibiting yeast growth. These are the off-flavours associated with beer contamination. These Veillonellae organism have been reported to grow in beer containing ≤5% (wt/vol) ethanol at pH ≥4.3 according to (Quain, 2001). Similar to gut bacteria, these bacteria may be introduced into beer through association with pitching yeast, causing product spoilage before ethanol and pH reach inhibitory levels, and pitching again, resulting in future growth which may contaminate the batch. These instances of biotic spoilage only emerged in recent years when the rise of unpasteurized beer and improvements in bottling equipment led to lower levels of dissolved oxygen in packaged beer. Zymomonas species which were observed in the research are a problem in beers with added sugars. Able to grow at pH above 3.4 and alcohol content above 10%, this bacterium is resistant to isoalpha acids and produces acetaldehyde (a green apple flavour) and hydrogen sulphide to spoil beer and cause beer to spoil by giving off an odour of rotten egg. However, this bacterium is not a common contaminant of beer as it is unable to ferment maltose and maltotriose, the major carbohydrates in wort and beer (Back, 2005). Spoilage is limited to beer with other added sugars. B. Sucrose and fructose sugars were added to SV 21 and 30 that reported Zymomonas bacteria (Bamforth, 2002). These brands have external sugars that are added in them to ensure enough sugars are available for the yeast to feed on.

Identified at only one instance before pasteurisation is a sign that they are extremely sensitive to temperatures greater than 10°C. Any organism that has not been introduced to a beer by the brewer is considered a spoilage organism because of its properties that could cause spoilage to the beer. Thus, the principal form of wild yeast contamination in beer is from rogue strains of *Saccharomyces cerevisiae* (Quain, 2001). These spoil beer through ester or phenolic off-flavour production (POF), formation of haze or sediment, or super-attenuation, leading to over-carbonation and diminished body, again these properties are shown in Boulton and Quain book (Quain, 2002). In *Saccharomyces* and other yeasts, POF is caused by decarboxylation of *p*-coumaric acid and ferulic acid to 4-vinylphenol and 4-vinylguaiacol, respectively, a property engendered by the *POF1* gene (T, 2008). These compounds give beer an unusual medicinal or spicy clove aroma and are atypical for most beers, though they are considered a marker trait of German wheat beers and some Belgian ales, as the yeasts used in these

beers are POF positive unfortunately, tasting these samples was not done. With regards to pasteurisation, these are extremely sensitive to temperatures and after pasteurisation, all species were reduced to zero as seen in the data collected at the end of this research. Wild yeast are nefarious contaminants of most beers and other alcoholic beverages, though their presence is often encouraged in other types of beer (Gilliland, 1967). These yeasts spoil beer through the production of the highly volatile phenolic compounds 4-ethylguaiacol and 4-ethylphenol, lending the aroma of bandages, sweat, and smoke. A number of other metabolites, including copious acetate production in the presence of oxygen (Back, 2005), result in a wide range of off-flavors produced by these yeasts.

A large number of other non-*Saccharomyces* yeasts are capable of growth in beer, but their spoilage potential is limited under optimal storage conditions, due to the combined factors of oxygen limitation, ethanol toxicity, and competition with *Saccharomyces* as seen before the beer was pasteurized. Further identification of these wild yeast was not done due to the fact that wild yeast are not expected in the brewery. They are a sign of contamination during the beer production process (T, 2008). Most of these yeasts spoil beer through the production of off-flavors (especially organic acids and POF), haze, sediment, or surface films (T, 2008). Like AAB, these yeasts are common throughout breweries, especially in unwashed sampling ports and on other surfaces contacting beer. The fact that they did not appear after pasteurisation means we do not dwell much on them.

5.2 Packaging and Distribution

Beer presents the two biggest challenges to beer microbial stability. In all previous brewing processes, from wort boiling to cold conditioning, wort and beer are placed in seamless stainless steel vessels that are extremely easy to clean (using state-of-the-art equipment and sanitary practices) assuming that. However, during packaging, the fresh product moves over the complex surfaces of the bottling plant, is briefly exposed to the atmosphere, and breaks down into smaller containers. Biofilms can form on the fill head surface and fill area, increasing the risk of microbial contamination. Casks are constantly reused, often cycled between different breweries and contain complex surfaces that are sealed and present a certain risk. Flash pasteurization must therefore be 100% effective without the transmission of micro-organisms to the beer. Casks returned to the brewery may be exposed to questionable conditions, including prolonged exposure to high temperatures and air.

The moment beer leaves the brewery, it is beyond the control of the brewer and subject to all conditions imposed by distributors, retailers and consumers. Packages can be exposed to temperature changes, light and/or turbulence, all of which affect the quality of the product inside besides exposure to light encourage microbial growth. Even under optimal storage conditions, it can take weeks of transportation and storage before significant amounts of beer are consumed, and given the size and distance of today's local beer distribution, the risk of microbial spoilage is high, more likely. The industry has long addressed this issue through product stabilization through filtration, pasteurization, or a combination.

However, the increased demand for unpasteurized beer in recent years has increased the incidence of microbial contamination of packaged beer with microorganisms such as *Pectinatus* that were identified in the research.

Beer distribution systems pose a particular threat to beer stability. This is because the dispensing mechanism itself involves the introduction of foreign matter into the packaging on site as stated in the literature review. After that, its stability is distributed in places (pubs, restaurants, or private apartments). The coupling penetrates the container, gas flows into the container and beer flows out. Compressed carbon dioxide enters the vessel, maintains the proper level of carbonation, and drives the beer through the tap line, taps and beverage containers, this was observed in the flash pasteurized beer. The compressed gas itself is sterile, but if not properly cleaned and sterilized, microorganisms can be introduced directly into the kegs via the gas lines and couplings. Beer distribution equipment (couplings, lines, and taps) encompasses a large surface area in contact with beer and contains many intricate surfaces that resist cleaning. Biofilms can hypothetically form along these surfaces, particularly in microcracks in sampling lines and crevices in pipetting equipment. These biofilms can support the survival of microorganisms not normally found in beer, but the composition of draft beer biofilms remains to be elucidated. Beer experiences a certain residence time in this uncooled environment before being delivered to the next customer. During this time, microbial cells trapped in the line proliferate and can cause turbidity, off-flavours and even spoilage through BA production.

However, results showed that bottled beer was prone to consistent aerobic bacterial contamination. Only Batch 2 showed a decrease in aerobic bacteria in the bottle and an increase in aerobic bacteria during flash sterilization.

In this study of pasteurized draft and bottled beer in Zambia, of all the beers tested (n=4), only the flash pasteurized (all four) contained dangerous levels of aerobic bacteria (>10cfu/ml). The observed results suggested that flash pasteurized beer should be avoided by those sensitive to BA which are by-products produced by LAB. Following good hygiene practices, including cleaning and disinfecting, regularly changing all tubing, and proper storage conditions.

With the results discussed, it is correct to say that the brewhouse, flash, and tunnel pasteurization processes mentioned are not effective. The pasteurization process is not effective before or after pasteurization. For this reason, breweries can adopt a number of practices that can improve the effectiveness of this sterilization process in order to reduce the microbial load of beer.

CHAPTER SIX

6.1 CONCLUSION AND RECOMMENDATIONS

The results of the current study indicated that the general quality and characteristics of beer are unstable. The experiment's findings provided a variety of insights into the various microbes connected to beer. Acetobacter is one of the microorganisms linked to the production of beer. Pediococcus, Lactobacillus, Zymomonas, Gluconobacter, Pectinatus and Pediococcus. This study's primary goal was to evaluate the impact of flash and tunnel pasteurisation on the quantity and quality of microorganisms, which entails comprehending their characteristics both before and after pasteurisation. Both systems' analyses of the beer following pasteurisation revealed residual microbiological contamination. According to the standards set by the literature review and its findings, flash pasteurisation is not as effective as previously thought. As of right now, the brewery is producing beer that tests positive for microbiological contamination, which may have implications for drinkers. The presence of microorganisms in the beer is a direct result of inadequate vessel cleaning and condition.

Recommendation: The dilution water used tested positive for microorganisms, which was discovered after more research to determine why bright beer is always positive for microorganisms. The brewery's dilution system's CIPs should be put into practice. Additionally, the BBTs' CIPs to Filler will begin. Identifying the hose management at filtration will require creating a SOP. However, over pasteurisation causes beer to go bad, so there must be a sweet spot. One of the challenges is that micro results are released after beer has hit the market. It is adviseable to use instantaneous swabs.

REFERENCES

1. Back, W (1997). *Technical and technological prerequisites for 'cold sterile' bottling*. BrauweltInternational, 15: 192–201.
2. Back, W. (2005). *Brewery. In: Colour Atlas and Handbook of Beverage Biology*. W. Back, Ed.Verlag Hans Carl, W. Pp. 10-112.
3. Back, W. (2009*). Microflora in the Brewery. In: Handbook of Brewing, Process, Technology, Markets*. Pp. 477-490.
4. Back, W (1994). *Secondary contamination in the filling area*. Brauwelt International.12(2):56
5. Bamforth, C. W (2002). *Standards of Brewing -A Practical Approach to Consistency and Excellence. Journal of the Institute of Brewing*. 108 (4): 514–515.
6. Bamforth, H. A. (2009). *Microbiological Stability of Beer: In Beer Quality Perspective.*
7. Boulton, C and Quain, D (2001). *Brewing Yeast and Fermentation*.Blackwell Publishing Ltd. Pp.143-259, 510-532.
8. Briggs, D. E., Boulton, C. A., Brookes, P.A and Stevens, R (2004). *Microbiology In: Brewing Science and Practice*.Whoodhead Publishing and CRC Press. Pp.567-575.
9. Briggs. (2004). *Microbiology in: brewing science and practice*. whoodhead Publishing.
10. Calina C, e. a., 2022. Beer Safety. *New Challenges and future trends with craft and large scale production.*
11. C, R. (1978). *Beer Spoilage Microorganisms In Brewing Science.*
12. C, R. (1981*). Beer Spoilage Microorganisms in Brewing Science*.
13. Campbell, P. a. (1996). *Brewing Microbiology*.
14. Chelack, G. J and Ingledew, W. M (1987*). Anaerobic Gram-Negative Bacteria in Brewing—A Review*. American Society of Brewing Chemist. 89.
15. Coint, C., 2022. Beer Safety. *Beer Safety: New Challenges and Future Trends within Craft and Large Scale Production.*
16. Crozier-Dodson, B and Fung, D (2002*). Comparison of recovery of airborne microorganisms in a dairy cattle facility using selective agar and thin layer resuscitation media*. Journal of Food Protection . 65(9):1488-1492.
17. Dagan, G. F. (2006). *Pasteurization of Beer by continous Dense-phase CO2 System*.
18. Dammann. (2011). *Flash Pastuerization of Beer; a critical reveiw.*
19. Deak. (2008). *Handbook of Food spoilage yeasts*. CRC Press. Taylor and Francis Group. Pp. 152-158.
20. Dirksen, J (2005*). Monitoring and controlling microbial contamination in the beer filling area*. MBAA Technical Quarterly, 42: 39-44.

21. Dirksen. (2005). *Monitoring and controlling microbial contamination in the beer filling area*.

22. Embashu, W. (2018). *Namibian Opaque Beer. A Reveiw*.

23. Encyclopedia, B. (October 2023). *Britannica, The Editors of Encyclopaedia. "Anheuser-Busch Companies, Inc.". Encyclopedia Britannica, 25 Oct. 2023, https://www.britannica.com/topic/Anheuser-Busch-Companies-Inc*.

24. Fernandez, J. L and Simpson, W. J (1993). *Aspects of the resistance of Lactic Acid Bacteria to hop bitter acids. Journal of Applied Microbiology*. 10(2):213-217.

25. Fernandez, s. (1993). *Aspects of resistence of Lactic Acid Bacteria to hop bitter acids*. *Allied Microbiology*.

26. Fleet, G. (1992). *Spoilage yeasts. Critical Reviews in Biotechnology*. 12: 1-44.

27. Gilliland. (1967). *Wild yeast spoilage. Brewery Guardian*. 96:37-45

28. Haikara, A and Helander, I. (2002). *Pectinatus, Megasphera and Zymophilus. In: Dworkin M, The Prokaryotes: An Evolving Electronic Database for the Microbiological Community*, 3rd edition.Finland new york. Pp. 111-116.

29. Haikara, A and Henriksson, E. (1992). *Detection of airborne microorganisms as a means of hygiene control in the brewery filling area*. 22nd Convention of the Institute of Brewing, Australia and New Zealand Section, Melbourne. Pp. 159-163.

30. Haikara,A and Lounatmaa, K.(1987*). Characterization of Megasphaera sp., a new anaerobic beer spoilage coccus* .In Proceedings of the European Brewer Convention,21°C,NewYork:Oxford University Press. Pp.473- 480

31. Haikara. (1984). *Beer Spoilage, Occurence and detection with the Genus Pectinatus*. , Technical Research Centre of Finland, Publication 14.

32. Helander, I. M., Haikara, A., Sadovskaya, I.,Vinogradov, E. and Salkinoja-Salonen, M. S. (2004). *Lipopolysaccharides of anaerobic beer spoilage bacteria of the genus Pectinatus – lipopolysaccharides of a Gram-positive genus. FEMS Microbiology Reviews* 28: 543–552.

33. Henriksson, E and Haikara, A (1991). *Airborne microorganisms in the brewery filling area and their effect on microbiological stability of beer*. Monatsschrift fur Brauwissenschaft, 44(1):4-8.

34. Hill, A.E and Bamforth C. W.(2009). *Microbiological stability of beer. In: Beer Quality Perspective*. Academic Press Elsevier. p .6o.

35. Hornsey. (2004). *A History of Beer and Brewing*. 1st edition.Washington D.C.: Royal Society of Chemistry. Pp.630-635.

36. Jakobsen, J. a. (1996). *Specificc spoilage organisms in breweries and Laboratory media for their detection.*

37. Janousek. (2001). *Effect of Tunnel Pasteurization upon flavour Stability of Beer*.

38. Jerpersen and Van, V. a. (1998). *Detectio in Larger Breweriesn and Identification of Wild Yeast.*

39. Jespersen, L and Jakobsen, M (1996). *Specific spoilage organisms in breweries and laboratory media for their detection*. International Journal for Food Microbiology, 33:139-155.

40. Juvonen, R. (2009) *DNA-based detection and characterisation of strictly anaerobic beer-spoilagebacteria.* VTT Technical Research Centre of Finland. Publication. 723.

41. Konings, S. (2003). *Beer spoilage bacteria and hop resistance.*

42. Kretsch, J. (1994). *Pratical considerations for brewery sanitation.* Mast. Bew. Ass. Amer. Tech.Quart., 31:124 - 128.

43. Loeffler. (2006). *Modern Brewery Sanitation.* In: C. W. Bamforth. Brewing, New technologies.Woodhead Publishing Limited, Abington Hall, Abington. Pp. 543 -549.

44. Lounatmaa, H. H. (1987). *Characterization of Megasphaera sp: a new anaerobic beer spoilage coccus.*

45. Manzano, M., Giusto, C., Bartolomeoli, I., Buiatti, S and Comi, G. (2005). *Microbiological Analyses of Dry and Slurry Yeasts for Brewing*. Journal International of Brewery,111(2): 203–208.

46. Maugueretand, T. M., Walker, S.L. (2002). *Rapid detection of Obesumbacterium proteus from yeast and wort using polymerase chain reaction. Letters in Applied Microbiology*, 35: 281–284.

47. McGovern, P. E., Zhang, J. Z and Tang, J. G. (2004) *Fermented beverages of pre- and proto-historic China*. Proceedings of the National Academy of Sciences, USA,101:17593–17598.

48. Miller, N (2012). "*The Beer Nut: Mikkeller brews beer on the run*". Pp.59-62. In:Priest F.G. and Campbell I. Brewing Microbiology 2nd edition.Pp.2245-2250

49. Novellie, L., 1986. *Modern Developments in Traditional African Beers*.

50. Nyamunda, B., 2017. *Extension of shelf life of commercial opaque beer using flash.*

51. Ouwehand, S. V. (2004*). Lactic Acid Bacteria: Microbiological and Functional Aspects*.

52. Paradh Ashtavinayak (2017). *A Review: Gram Negative Bacteria In Brewing.* Department of Alcohol Technology, Vasantdad Sugar Instituite, Pune, India.

53. Priest F. G. and Campbell I. (1996). *Brewing Microbiology* 2nd edition. Chapman and Hall.Pp. 1056-1058.

54. Priest F.G. and Campbell I. *Brewing Microbiology* 2nd edition. Pp.2245-2250

55. Priest, F. G (1996). *Gram-positive brewery bacteria*.

56. Priest, F. G and Stewart, G. G.(2006). *Microbiology and Microbiological Control in the Brewery*.16. 607-626. In: Handbook of Brewing. CRC Press Taylor and Francis Group,16: 607-626.

57. Priest. (1996). *Brewing Microbiology*. 2nd edition. Chapman and Hall.Pp. 1056-1058.
58. Quain, B. a. (2001). *Brewing Science.*
59. Quain, B. a. (2002). *Brewing Yeast and Fermentation*. Blackwell Publishing.
60. Rainbow, C (1981). *Beer spoilage microrganisms. In: Brewing Science, vol II*, J.R.A. Pollock.
61. Ryder, D. S., Murray, J. P and Stewart, M (1978*). Phenolic off-flavour problem caused by Saccharomyces wild yeast*. MBAA Technical Quarterly,15:79-86.
62. Sakamoto, K and Konings, W (2003*). Beer spoilage bacteria and hop resistance*. InternationalJournal of Food Microbiology 89. 105-124.
63. Salminen, S., Von Wright, A and Ouwehand, A. (2004). *Lactic Acid Bacteria. Microbiological and Functional Aspects*. 3rd Edition, Marcel Dekker, Incoperation.Pp.689 -700.
64. Sarlin, T., Nakari-Setala, T., Linder, M., Penttila, M and Haikara, A (2005*). Fungal hydrophobins as predictors of the gushing activity of malt. Journal of the Institute of Brewing*, 111: 105 – 111.
65. Schleifer, K.H., Leuteritz, M., Weiss, N., Ludwig, W., Kirchof, G.and Seidel-Rufer, H. (1990) *Taxonomic study of anaerobic, gram-negative, rod-shaped bacteria from breweries: emended description of Pectinatus cerevisiiphilus a description of Pectinatus frisingensis sp., Selenomonas lacticifex sp. nov., Zymophilus raffinosivoransgen. nov., sp. nov., andZymophilus paucivoran ssp.nov.*. International Journal System Bacteriology. 40: 19–27.
66. Sharpton, D., 2006. Identification Methods for Microbiologists Part B.
67. Stewart, G. G and Russell, I (1998*). An Introduction to Brewing Science and Technology Series III: Brewer's Yeast.*The Institute of Brewing. London, England. Pp. 23-31.
68. Stewart, P. a. (2006). *Microbiology and Microbiological Control in the Brewery.*
69. Storgårds, E., Haikara, A. and Juvonen, R. (2006). *Brewing control systems: microbiological analysis*. In:Bamforth C.W. Brewing. New technologies.
70. Storgards, E. (2000). Process hygiene control in beer production and dispensing. *Process hygiene control in beer production and dispensing*. Technical Research Centre of Finland
71. Suzuki. (2011). *Microbiological Instability of Beercaused by Spoilage Bacteria*. Journal of the Institute of Brewing, 117(2):131-155.
72. Tamime A. Y. (2008*). Cleaning-in-Place. Dairy Food and Beverage Operations*. Blackwell Publishing Ltd.Pp.39 - 43.
73. Thelen et al, T. e. (2006). *Evaluation Study of the Frequency of Different Beer Spoiling Bacteria using VIT analysis.*

74. Thelen, K., Beimfohr, C. and Snaidr, J. (2006). *Evaluation study of the frequency of different beer spoiling bacteria using the VIT analysis*. Master Brewers Association of the Americas Technical Quarterly, 43: 31-35.
75. Thurston, P. A. (1986). *The phenolic off-flavour test: A method for confirming the presence of wild yeasts*. Journal of the Institute of Brewing, 92: 9-10.
76. Van Vuuren, H. J. J and Priest, F. G (1996). *Gram-negative brewery bacteria*. In: Priest, F.G, and Campbell, I. Brewing Microbiology 3rd edition. Pp.2251- 2253 .
77. Van der Kühle, A and Jespersen, L. (1998). *Detection and identification of wild yeast in lager breweries*. Journal of the instutite of brewery. 112-120.
78. Vanbeneden N., Frederik, G., F, D and Freddy, R. D (2008). *Formation of 4-vinyl and 4-ethyl derivatives from hydroxycinnamic acids: Occurrence of volatile phenolic flavour compounds in beer and distribution of Pad1-activity among brewing yeasts*. Food Chemistry. 107
79. Vaughan, A., O'Sullivan, T. and Van Sinderen, D. (2005). *Enhancing the Microbiological Stability of Malt and Beer*. Journal of the Institute of Brewing. 111(4):335-371.
80. Vaughan. (2005). *Enhancing The Microbiological Stability Of Malt and Beer*.
81. W, B. (1994). *Secondary Contamination in the filling Area.*
82. W, B. (2020). *Brewing Techniques in Practice*.

APPENDIX I : RESEARCH BUDGET

NO.	ITEM DESCRIPTION	QUANTITY	UNIT TOTAL (ZMW)	TOTAL (ZMW)
1	Sterile sampling kit	24	150	3600
2	SSSB Bottles	8	50	400
3	NBB A Agar	1	1700	1700
4	NBB B Agar	1	1690	1690
5	NBB C Agar	1	1690	1690
6	PCA	1	2010	2010
7	Copper Sulphate Agar	1	1800	1800
8	WLN	1	1250	1250
9	Yeast and Mould Agar	1	1970	1970
10	Distilled water	2	50	100
11	Methylated Spirit	1	65	65
12	Petri Dishes	50	5	250

13	Stationary	1	500	500
14	90% ethanol	1	300	300
15	Gloves	1 box	200	200
16	Transport	1	1250	1250
17	Glass slides and cover slips	1	300	300
	GRAND TOTAL			19,075