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Kombucha Production in Uganda: Quality Aspects and Compliance with Standards

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DECLARATION

I, Rehema Meeme, hereby proclaim that, this thesis gives facts of the work I have accomplished during my research on Kombucha production in Uganda: focusing on the quality aspects and compliance with standards. This research thesis is backed-up with results from the laboratory and field as my individual input and devotion in completion of my final year Masters research at the School of Food Technology, Nutrition and Bioengineering, Makerere University.

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APPROVAL

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DEDICATION

I dedicate this research thesis to my family in recognition of the moral and emotional support they have always invested in me during my academic journey. I also dedicate my work to my academic supervisors: Assoc. Prof. Ivan Muzira Mukisa and Dr. Robert Mugabi for the endless encouragement and guidance accorded to me during my research work.

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DEFINITION OF TERMS

Beverage	A commercial drink other than water, intended for human consumption.
Critical Control Point	A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.
Critical Limit	A criterion which separates acceptability from unacceptability.
Food Safety	Assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use.
GHP	Good Hygienic Practices (GHP) refers to all practices regarding the conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain.
HACCP	Hazard Analysis and Critical Control Point (HACCP) is a system which identifies, evaluates and controls hazards which are significant for food safety in a given food segment.
HACCP Plan	A document prepared in accordance with the principles of HACCP to ensure control of hazards which are significant for food safety in the segment of the food chain under consideration.
Kombucha	A fermented, lightly sweet black or green tea drink commonly consumed for its purported health benefits.
Standards	Documents that contain technical and other requirements that products, services and systems have to comply with.

ABBREVIATIONS AND ACRONYMS

AAB	Acetic Acid Bacteria
CCP	Critical Control Point
CFU	Colony Forming Unit
CL	Critical Limit
CP	Critical Points
GHP	Good Hygienic Practices
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis and Critical Control Point
ISO	International Organization for Standardization
LAB	Lactic Acid Bacteria
MW	Molecular Weight
(n)	Frequency
OPRP	Operational Pre-requisite Program
SCOBY	Symbiotic Culture of Bacteria and Yeast
SDGs	Sustainable Development Goals
SMEs	Small and Medium Enterprises
SOPs	Standard Operating procedures
UNBS	Uganda National Bureau of Standards
US	Uganda Standard

ABSTRACT

Kombucha is a slightly sweet and acidic fermented tea beverage. It's production and consumption in Uganda is on the rise due to associated claimed nutritional and health benefits to the consumers for example ,it is reported to help prevent bladder infections, reduce kidney calcification, protect against diabetes and increase body's resistance to cancer.

There has been limited information on the quality and safety of commercially produced Kombucha in Uganda. This study evaluated knowledge and practices of Kombucha processors (n=8) with certified and uncertified products in Uganda. It also evaluated the quality and safety of certified (n=4) and uncertified (n=4) Kombucha. A Hazard Analysis and Critical Control Point plan for Kombucha processing was developed and validated.

Majority of the processors (n=6) had very good (scores above 75%) knowledge and practices related to food safety. Most processors (n=6) did not know the importance of sanitizing equipment. Half of the processors did not know about HACCP, its pre-requisites and Kombucha specification. Processors (n=4) did not use objectives methods to test product readiness. Half of the processors (n=4) did not follow the Kombucha specification while (n=5) had no HACCP plan. All products passed the Kombucha requirements for *Staphylococcus aureus, Escherichia coli, Salmonella* spp., acidity and heavy metals (lead, arsenic, mercury and cadmium). However, 75% of the products did not meet the quality and safety specifications for Kombucha. A HACCP plan with three CCPs and five CPs was developed and validated. Adoption of the HACCP plan resulted in the products complying with the yeast and molds requirements as specified in Kombucha specification (US 2037: 2019). Hence, adoption and implementation of the HACCP plan resulted in significant improvement of quality and safety of Kombucha.

This study, therefore, informs Kombucha processors and regulators on the safety and quality of Kombucha on the market, importance of HACCP plan development, training and implementation, which will lead to improved regulation and compliance with the relevant food safety standards in future.

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CHAPTER ONE

INTRODUCTION

1.1 Background

Kombucha is a slightly sweet and acidic refreshing beverage obtained by fermenting sugared black or green tea made from *Camellia sinensis* (L.) Kuntze leaves, with a consortium of yeast and predominantly Acetic Acid Bacteria (Jayabalan et al., 2014). This beverage is also often slightly alcoholic and lightly effervescent. Kombucha is consumed worldwide and is an emerging popular beverage in Uganda commonly consumed for its supposed health benefits.

Kombucha has also been defined by Jayabalan et al. (2014) as a tea mushroom, tea fungus, or Manchurian mushroom. In deed sometimes, the beverage is called Kombucha tea to distinguish it from the culture of bacteria and yeast as noted by Mayo Clinic (2018). Besides sugared tea, other substrates, such as juices, spices, fruit or flavorings are often added to enhance the taste of the beverage (Leal et al., 2018).

Kombucha is thought to have originated in Manchuria, China where the drink is traditionally consumed, or in Russia and Eastern Europe (Jayabalan et al., 2014; Troitino, 2017). Kombucha is now homebrewed or commercially brewed globally, bottled and sold by various companies (Jayabalan et al., 2014).

Kombucha is produced by fermenting sugared tea using a symbiotic culture of bacteria and yeast (SCOBY) commonly called a "mother" or "mushroom" (Leal et al., 2018). Although the SCOBY is commonly called tea fungus or mushroom, it is actually a symbiotic growth of acetic acid bacteria (AAB) and osmophilic yeast species in a zoogleal mat (biofilm) composed of cellulose (Jayabalan et al., 2014). According to Jonas and Farah (1998), the microbial populations in a SCOBY vary. The yeast component generally includes *Saccharomyces cerevisiae*, along with other species such as *Candida*, *Pichia*, *Brettanomyces*, *Saccharomyces*, *Lachancea*, *Saccharomycoides*, *Schizosaccharomyces*, and *Kluyveromyces* (Jayabalan et al., 2014). The yeasts produce ethanol and some flavor compounds for example; D-glucuronic acid, citric acid, L-lactate, Benzeneacetaldehyde and acetic acid (Villarreal-Soto et al., 2018). The bacterial component usually includes *Gluconacetobacter xylinus/Komagataeibacter xylinus/ Acetobacter xylinum,*

which oxidizes yeast-produced alcohols to acetic acid and other organic acids, thus increasing the product acidity and limiting ethanol content (Greenwalt et al., 2000). *Komagataeibacter Xylinus* is also responsible for production of cellulose (from sugars and ethanol) resulting in formation of a pellicle in which the Acetic Acid Bacteria and yeasts are embedded (Villarreal-Soto et al., 2018). Other species of bacteria reported in Kombucha include Lactic Acid Bacteria (LAB) genera such as Lactobacillus and Lactococcus (Villarreal-Soto et al., 2018).

Commercially bottled Kombucha became available in the late 1990s (Wollan, 2010). In 2010, elevated alcohol levels were found in many bottled Kombucha products. Leading retailers included; Whole Foods Market, USA, temporarily pulled the drinks from store shelves (Rothman, 2013). In response, Kombucha suppliers reformulated their products to have lower alcohol levels (Crum, 2011). However, as of 2019, some commercial Kombucha producers sell 'hard Kombucha' with an elevated alcohol content of 4.5% (Casey, 2019; Judkis, 2018).

By 2014, sales of bottled Kombucha in the United States of America were $400 million, $350 million of which was earned by Millennium products, international (Narula, 2015). In 2014, companies in the US that make and sell Kombucha formed a trade organization called Kombucha Brewers International. PepsiCo in 2016 purchased Kombucha maker KeVita for approximately $200 million.

Kombucha is also becoming popular in Uganda although there is limited information on the sales and consumption data available. There are number of ‘known’ and ‘unknown’ companies producing Kombucha in the country at small, medium and large scale. In this sense ‘known’ companies are registered companies whose products are certified by the Uganda National Bureau of Standards (UNBS). As of 17 August 2020, the ‘known’ Kombucha certified products in Uganda were Twenty-five (25) in total (UNBS, 2020). The quality and safety aspects of Kombucha include; microorganisms of concern such as Yeasts and molds, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp., heavy metals (Lead, Cadmium, Arsenic, Mercury), alcohol content and acidity as acetic acid (UNBS, 2019).

Kombucha consumption has been associated with several health benefits (Ernst, 2003; Jayabalan et al., 2014). Most of the benefits have not been demonstrated in human trials but done *in vitro* or in animal studies. Non-human studies regarding antimicrobial, antioxidant, hepatoprotective, and anticancer properties of Kombucha have been carried out (Gharib, 2014; Ernst, 2003). However,

there is insufficient scientific evidence based on human models. These claims among others include; detoxifying blood, reducing blood cholesterol levels, reducing atherosclerosis by regeneration of cell walls, reducing blood pressure and inflammatory problems (Gharib, 2014; Ernst, 2003). Others include alleviating arthritis, rheumatism, and gout symptoms on top of promoting liver functions, normalizing intestinal activity and balancing the intestinal flora (Ernst, 2003). Kombucha cures hemorrhoids, reduces obesity and regulates appetite (Ernst, 2003). The beverage is also associated with preventing bladder infection and reducing kidney calcification. It also reportedly stimulates glandular systems, protects against diabetes, increases body resistance to cancer, and reduces an alcoholic's craving (Wang et al., 2010; Ernst, 2003). Additionally, Kombucha reduces stress, lowers nervous disturbances, lessens insomnia and relieves headaches (Ernst, 2003; Jayabalan et al., 2014).

There are also several potential risks that have been associated with Kombucha consumption (Villarreal-Soto et al., 2018). For example, internal lesions on the organs of rats after twelve weeks' feeding trial. However, mice given the same treatment had no ill effects (Villarreal-Soto et al., 2018). The study noted susceptibility to toxicity from Kombucha varies from species to species. Another potential risk is food poisoning in case of pathogens getting into the product because of poor hygiene (Villarreal-Soto et al., 2018). Excess intake of the beverage could also result in acidosis (Dasgupta, 2013; Nummer, 2013). Greenwalt et al., (2000) also noted that excessive intake of Kombucha is associated with the risk of liver damage. Liver damage could be connected to the cumulative effect of intake of small amounts of alcohol (0.7-1.3%) contained in the product (Kapp and Summer, 2019). Other potential risks of consuming Kombucha are associated with a low pH brew leaching heavy metals from containers, excessive consumption of highly acidic Kombucha, or consumption by individuals with pre-existing health conditions (Kapp and Sumner, 2019). Kombucha is, therefore, contraindicated in pregnant women and people with significant renal, pulmonary, or liver disease (Kapp and Sumner, 2019). Despite these implications, Kombucha is not considered harmful if about 120 ml per day is consumed by healthy individuals (Kapp and Sumner, 2019; Martini, 2018).

1.2 Problem statement

There is increasing interest in the consumption of safe and healthy foods such as fermented beverages which are associated with nutritional and healthy benefits. Kombucha is a fermented food product with high commercial potential due to its numerous claimed health benefits. Kombucha is currently locally produced in Uganda and is highly consumed by people of different age groups. Most Kombucha products on the Ugandan market are processed by Small and Medium Enterprises (SMEs) under several brands (***Appendix 3***).

In Uganda, just like in many developing countries, most SME's are slow at adopting advanced processing technologies. Some SMEs have underdeveloped food safety control systems and often do not comply with recommended good manufacturing practices. This is worsened by the fact that most local SMEs are run by unqualified personnel with little knowledge in food processing, quality, safety and hygiene. The end-result is that, most SMEs frequently fail to meet product specifications and other relevant standards. This could inadvertently contribute to the burden of food borne illnesses arising from pathogens like *Staphylococcus aureus* and *Escherichia coli*.

Prior to this study, there was limited information on the quality and safety of Kombucha on the market in Uganda. The little information available indicated that Kombucha was mainly produced by fermenting sugared black tea for up to 14 days. The major stages in Kombucha production which could compromise the product safety and quality include; Raw material reception and storage, cooling of boiled mixture of sugar, tea leaves and water prior to fermentation, fermentation stage, packaging of ready Kombucha and storage and distribution of Kombucha products. The knowledge and practices of the processors which are crucial for product safety had prior to this not been evaluated. Little or no information about the compliance of commercial Kombucha processors with recommended quality and safety management practices/systems was available.

This study, therefore, established the quality and safety of Kombucha on the market in Uganda. It also evaluated the knowledge and practices of the Kombucha processors with certified and uncertified products on the market. The study further established the food safety gaps in Kombucha production and proposed a Hazard Analysis and Critical Control Plan (HACCP) that can be modified and adopted by SMEs to ensure product quality and safety.

1.3 Objectives

1.3.1 General objective

To evaluate the quality of Kombucha on the market in Uganda and develop a HACCP plan to enhance its safety.

1.3.2 Specific objectives

1. To evaluate the knowledge and practices of Kombucha processors with certified and uncertified products in Uganda
2. To evaluate the quality and safety of certified and uncertified Kombucha on the market in Uganda
3. To develop and validate a Hazard Analysis and Critical Control Point plan for Kombucha processing

1.4 Research hypotheses

1. Ha1: There is a relationship between Kombucha product certification and the knowledge and practices of Kombucha processors
2. Ha2: There is a relationship between Kombucha product certification and the quality of Kombucha
3. Ha3: Adopting a HACCP plan improves the safety and quality of Kombucha

1.5 Significance of study

The beverage market in Uganda is one of the biggest sections of foods and beverage trade. This could explain why the Kombucha industry and market in Uganda has grown rapidly. Foods and beverages can serve as vehicle for food borne illnesses, especially if their processing is unregulated and unstandardized. This study will inform processors and regulators on the safety and quality of Kombucha on the market. This will lead to developing mechanisms for improving quality and safety of Kombucha. Ultimately, consumers will be protected from consumption of sub-standard and unsafe foods that could cause illnesses. The study is also in line with SDG (3) that focuses on ensuring healthy lives and promoting wellbeing for all. Thus, this study will suggest quality assurance mechanisms, which will enable the consumers, enjoy safe and quality Kombucha.

CHAPTER TWO

LITERATURE REVIEW

2.1 Description and history of Kombucha

Kombucha is a slightly sweet and acidic refreshing beverage consumed worldwide. It is obtained from an infusion of sugared tea leaves that is fermented by a symbiotic association of Acetic Acid Bacteria and osmophilic yeasts (SCOBY) also known as the "tea fungus" (Chen and Liu, 2000). A floating cellulosic pellicle layer (SCOBY) and sour liquid broth are the two main portions of Kombucha. Kombucha tastes like sparkling apple cider and produced at home by fermentation process using available tea fungus. Chen and Liu, (2000) further noted that, although green tea can be used for Kombucha preparation, black tea and white sugar are considered the finest substrates.

According to Jayabalan et al., (2014), Kombucha is the internationally used Germanized form of the Japanese name for this slightly fermented tea beverage, it was first used in East Asia for its healing benefits. Jayabalan et al., (2014) further narrated that, Kombucha originated in northeast China (Manchuria) where it was prized during the Tsin Dynasty ("Ling Chi"), about 220 B.C., for its detoxifying and energizing properties. In 414 A.D., the physician Kombu brought the tea fungus to Japan and he used it to cure the digestive problems of the Emperor Inkyo. As trade routes expanded, Kombucha (former trade name "Mo-Gu") found its way first into Russian (as Cainiigrib, Cainii kvass, Japonskigrib, Kambucha, Jsakvasska) and then into other eastern European areas appearing in Germany (as Heldenpilz, Kombucha schwamm).

Jayabalan et al., (2014) further noted that Kombucha was again introduced into Germany during World War II. This beverage arrived in France in the 1950's and it spread to France-dominated North Africa where its consumption became quite popular. The habit of drinking fermented tea became acceptable throughout Europe until World War II, which brought widespread shortages of the necessary tealeaves and sugar. Italian society's passion for the beverage (called "Funkochinese") peaked in the 1950s, then during 1960s science researchers in Switzerland reported that drinking Kombucha was similarly beneficial as eating yogurt (Jayabalan et al., 2014).

Kapp and Sumner, (2019) also reported Kombucha to have originated in northeast China about 220 B.C., disseminated to Japan in 414 AD as a medicine, and spread through trade routes to

Russia and eastern Europe. Recognizing the growing market, in 2016, PepsiCo purchased KeVita, a popular functional probiotic and Kombucha beverage maker. Kapp and Sumner, (2019) continued to note that, in 2017, retail sales of Kombucha and other fermented beverages increased by 37.4%. Kombucha is reportedly the fastest growing product in the functional beverage market and one of the most popular low-alcoholic fermented beverages in the world.

Ozdemir and Con (2017) explained that, this fermented tea has been produced and consumed in the Far East for hundreds of years as a tradition, it is used as an adjunctive treatment for certain diseases. The interest in this tea has increased recently, and Kombucha has spread worldwide. Its microflora diversity and metabolites are becoming important subjects of scientific studies.

Ozdemir and Con (2017) reported that, the liquid portion of Kombucha is claimed to have various medicinal effects on human health. Ozdemir and Con (2017) mentioned that Kombucha prevents paracetamol-induced hepatotoxicity and chromate induced oxidative stress in albino rats. As Kombucha is rich in compounds known to be strong antioxidants that ameliorates liver damage. The beneficial effects of Kombucha are attributed to the presence of tea polyphenols, gluconic acid, glucuronic acid, lactic acid, vitamins, amino acids, antibiotics and a variety of micronutrients produced during fermentation (Ozdemir and Con, 2017).

2.1.1 Production of Kombucha

Kombucha is prepared by placing the Kombucha culture (tea fungus) into a sugared tea broth for fermentation. If the Kombucha culture is cultivated according to the standard recipe with black tea, sweetened with sucrose, it turns this substrate into a refreshing beverage called tea fungus with high nutritive value and medicinal properties (Lončar et al., 2000).

Jayabalan et al., (2014) noted that, the popularity of Kombucha expanded like many other traditional beverages due to its beneficial effects on human health and its ease in home preparation with the amounts of tea, sugar, and tea fungus differing in different places.

Jayabalan et al., (2014) explained a standard procedure for preparation of Kombucha as follows: water (1 L) is boiled and 50 g sucrose stirred during boiling, 5 g of tea leaves added and removed by filtration after 5 min. After cooling to room temperature (20 ºC) the tea is inoculated with 24 g of tea fungus (culture) and poured into a beaker (1 L) previously sterilized with boiling water. The pH of Kombucha is lowered by addition of 0.2 L of previously fermented Kombucha to inhibit

growth of undesirable microorganisms. The beaker is covered with a paper towel to keep away insects, especially drosophila fruit flies. The mixture is then incubated at 20 ºC to 22 ºC. In a few days, the newly formed daughter culture starts to float and form a clear thin gel-like membrane across the available surface. This is the newly formed tea fungus available as a new layer above the old tea fungus which was inoculated to begin the fermentation. At this time, the tea starts to smell fermented and gas bubbles appear from the carbonic acid produced during fermentation. The mother culture remains at its original volume as it sinks to the bottom of the tea broth where it remains under the newly forming daughter culture. After 10 to 14 days, a new tea fungus will have developed on the surface of the tea as a disc of 2 cm thickness covering the whole diameter of the beaker. The newly formed tea fungus is removed and kept in a small volume of fermented tea. The remaining beverage is filtered and stored in a capped bottle at 4 ºC.

Jayabalan et al., (2014) also mentioned that, the taste of Kombucha changes during fermentation from a pleasantly fruity sour-like sparkling flavor after a few days to a mild vinegar-like taste after a long incubation period. It is remarkable that 50 g sucrose/L provide the optimal concentrations of ethanol and lactic acid and this sugar concentration has been used in traditional recipes for the preparation of "teakwass" (another name for Kombucha) for a long time (Jayabalan et al., 2014).

An optimum fermentation time is required for the production of Kombucha with pleasant flavor and taste. Longer fermentation produces high levels of acids (like mild vinegar) that may pose potential risks when consumed (Sreeramulu et al., 2000).

Kombucha production procedure using the standard method is given in Figure 1 below (Jayabalan et al., 2014).

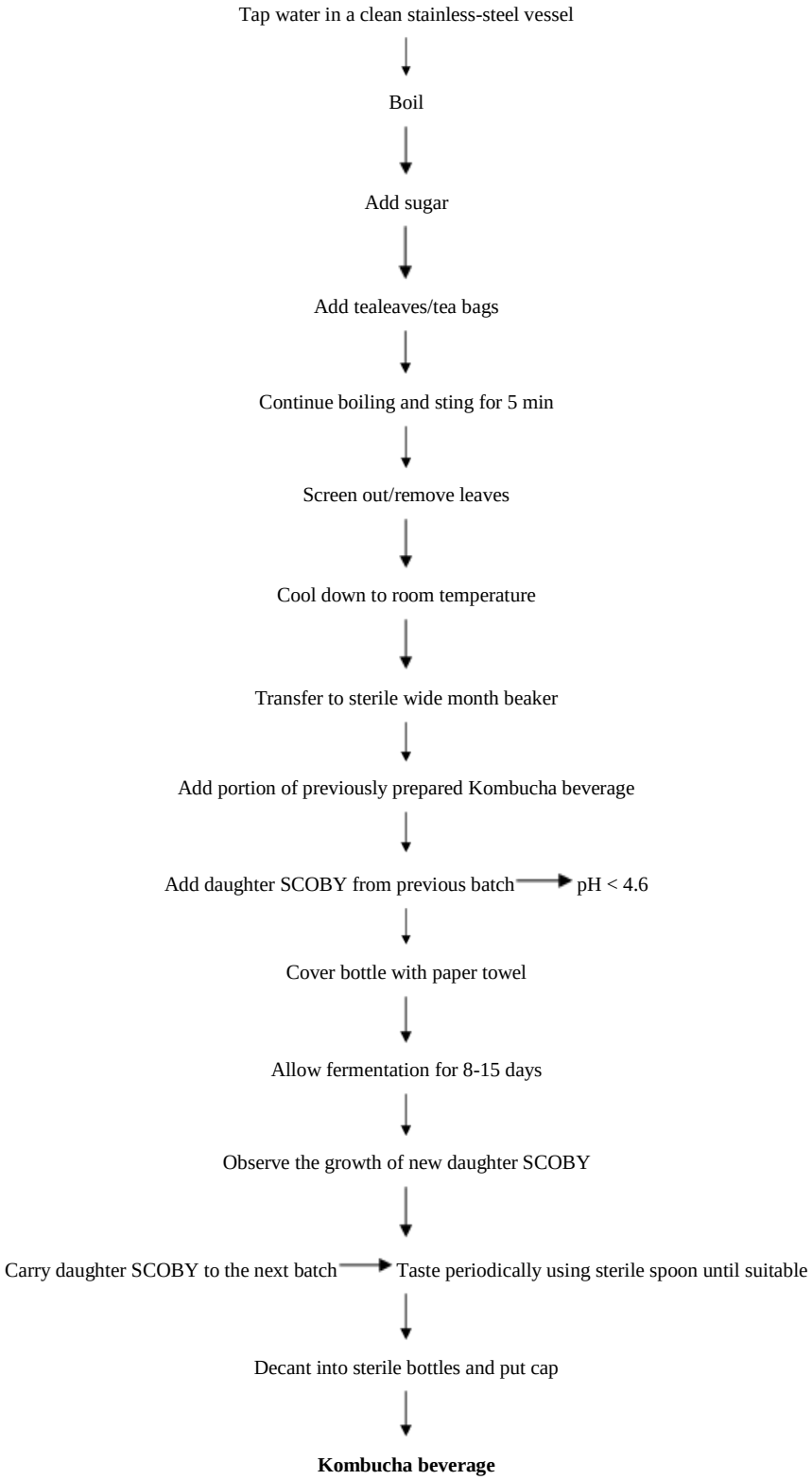

Figure 1: Process flow diagram for the production of Kombucha

Jayabalan et al., (2014) noted that a symbiotic culture of bacteria and yeast 'SCOBY' is key to Kombucha fermentation and consists of a cellulosic pellic layer that floats on the Kombucha's surface. Every SCOBY is a unique combination of Acetic Acid Bacteria and osmophilic yeast species that vary by climate, geography, culture, and wild microorganisms.

Jayabalan et al., 2(014) further noted that Acetic Acid Bacteria polymerize the glucose in the fermenting Kombucha solution to generate cellulose and hemi-cellulose as a secondary metabolites, forming the SCOBY's zoogleal mat. After 10 to 14 days of fermentation, a new SCOBY culture reaches 2 cm thick and separates from the inoculation mother culture. This new SCOBY is clear with a gel-like consistency. Once the fermentation is complete, the SCOBY can be saved in a small volume of Kombucha and used to inoculate new batches.

Commercial Kombucha brewing produces large volumes of SCOBY, as a new culture forms with every batch. SCOBY is an excellent animal feed as it contains high concentrations of crude protein, fiber, and lysine (Jayabalan et al., 2014). Studies have also shown that dried SCOBY can act as a bio-sorbent to collect metal pollutants from wastewater (Jayabalan et al., 2014).

Other researchers like (McHugh and Sinrod, 2019) also explained that, incomplete Kombucha fermentation produces Kombucha that lacks its characteristic flavor. However, fermenting Kombucha for too long increases the organic acid concentration to potentially harmful levels. The alcohol content also increases at the beginning of anaerobic fermentations. McHugh and Sinrod, (2019) further mentioned that beverages must contain less than 0.5% alcohol to be sold as nonalcoholic in the United States. If the sugar or yeast concentration is too high during fermentation or the Kombucha is fermented too long, the drink can reach 1.5% alcohol thus making alcoholic Kombucha. McHugh and Sinrod (2019) continued to report that while fermentation time depends on specific ingredients, their concentrations and environmental conditions a 7-day fermentation is considered the ideal time.

A standard method described by (Jayabalan et al., 2014) in Figure 1, is based on several other previously published reports on Kombucha by most Kombucha researchers (Malbaša et al., 2008; Morshedi and Dashti-Rahmatabadi, 2010; Mohammadshirazi and Kalhor, 2016).

Many modern Kombucha breweries have installed large-scale batch fermentation set-ups for its industrial-scale production. Although the domestic-scale method and conditions of fermentation

have remained almost the same. For large-scale processes, critical care should be taken for product safety in terms of clean and efficient water usage (Dutta and Paul, 2019).

Dutta and Paul, (2019) further noted that, sterilization of fermenter, and bottles should be ensured and avoiding use of hard water with dissolved minerals, salt, and alkali that may hamper the growth of the SCOBY and recommended use of sterilized deionized water.

Kombucha fermentation requires partial aerobic conditions and sufficient release of CO_2 formed during fermentation in order to produce the characteristic acids and metabolites (Dutta and Paul, 2019). A two-stage method has been described by several Kombucha makers in their blogs and reported to produce better quality beverage. In this, the daughter SCOBY is used for a second stage of the Kombucha fermentation. Alternatively, some follow a secondary fermentation technique in which the daughter SCOBY is removed and yeasts that remain suspended in the liquor allowed to ferment the material under anaerobic environment. This reportedly increases the dissolved CO_2 content in the beverage (Dutta and Paul, 2019).

It was also noted to use sanitized utensils and work in clean areas while making Kombucha, in order to have control over the growth of undesirable microorganisms and to prevent unwanted contamination (Watawana et al., 2015). Likewise, it is important to control pH levels during fermentation of Kombucha, and preferably stop the process when a pH level of 4.2 is reached, since the overproduction of acetic acid may be counterproductive (Kovacevic et al., 2014). Other food safety methods include pasteurizing the final product to prevent overproduction of alcohol and carbon dioxide, as well as the addition of 0.1% of sodium benzoate and 0.1% of potassium sorbate as food preservatives, and finally, keeping it refrigerated (Watawana et al., 2015).

2.1.2 The Kombucha culture

Tea fungus or Kombucha is the common name given to a symbiotic growth of Acetic Acid Bacteria and osmophilic yeast species in a zoogleal mat which has to be cultured in sugared tea (Jarrell et al., 2000). According to Jarrell et al., (2000), Kombucha is a consortium of yeasts and bacteria.

Sievers et al., (1995) reported that, the microflora embedded in the cellulose layer is a mixed culture of *Acetobacter xylinum* and *Zygosaccharomyces sp*. The predominant Acetic Acid Bacteria

found in the tea fungus are *Acetobacter xylium*, *Acetobacter pasteurianus*, *Acetobacter aceti*, and *Gluconobacter oxydans* as according to (Liu, 2013). Other reports noted that *Gluconacetobacter* spp. A4 which has strong ability to produce D-saccharic acid-1,4-lactone (DSL), was the key functional bacterial species isolated from a preserved Kombucha (Yang et al., 2010).

Some studies noted that, the tea fungus is not a mushroom but a SCOBY with a formal botanical name, *Medusomyces gisevii* (Jarrell et al., 2000). The name 'tea fungus' was wrongly given due to the ability of bacteria to synthesize a floating cellulose network which appears like surface mold on the undisturbed, unshaken medium (Jarrell et al., 2000). Similarly, to milk-derived kefir, the exact microbial composition of Kombucha cannot be given because it varies, it depends on the source of the inoculum for the tea fermentation. One of the clearer accounts of the microbes found in Kombucha starter is from *Acetobacter* spp. (Jarrell et al., 2000). Isolation of an *Acetobacter* spp. (NRRL B2357) and two yeasts (NRRL YB-4810, NRRL YB-4882) from a Kombucha sample received from Switzerland was done and microorganisms were used to produce Kombucha (Jarrell et al., 2000).

The most abundant prokaryotes in the culture belong to the bacterial genera *Acetobacter* and *Gluconobacter*, the basic bacterium being *Acetobacter xylinum* (Jarrell et al., 2000). It produces a cellulosic floating network on the surface of the fermenting liquid. The network is the secondary metabolite of Kombucha fermentation but also one of the unique features of the culture (Jarrell et al., 2000).

Martínez Leal et al., (2018) also mentioned that, the culture used for the Kombucha fermentation has a variable microbiological composition according to its origin, weather, geographical location and medium. Growth of this consortium of bacteria and yeasts induces the addition of new thicker membranes that take shapes of their container and heightens which is the symbiotic effect between bacteria and yeast (Martínez Leal et al., 2018). The cellulose membrane keeps the microorganisms on the surface, allowing enough oxygen availability for its development and protecting the microorganisms from UV rays (Suhartatik et al., 2011).

Several factors play an important role in the concentration of Kombucha constituents and one of them is temperature. According to the investigation done by Fu et al., (2014), keeping Kombucha refrigerated at 4°C mildly decreases the content of AAB, from 9.3×10^6 CFU/ml to 3.4×10^6

CFU/ml during 14 days of storage. The content of LAB decreases significantly, from approximately 23.5×10^6 CFU/ml to 2.7×10^3 CFU/ml during 8 days of storage.

It has been reported that yeasts have a positive impact on the survival of Lactic Acid Bacteria at 30°C, but not at 12°C (Suharja et al., 2014). This could mean that low cooling temperature of 4°C may have limitation on the positive effect of yeasts over Lactic Acid Bacteria, reducing their survival rate (Fu et al., 2014). The other microbes in Kombucha SCOBY are indicated in Table 1 below.

Table 1: Diversity of the microorganisms in Kombucha culture fermentation and their roles

SN	Microorganism	Role	Source
	Bactria		
1	*Acetobacter intermedius sp. nov* , *Acetobacter nitrogenifigens sp. Nov, Gluconacetobacter kombuchae sp. nov*	Nitrogen-fixing Cellulose-producing	Boesch, (1998) Dutta and Gachhui,(2007) Marsh, (2014)
	Yeasts		
2	*Saccharomyces cerevisiae* *Saccharomyces bisporus* *Saccharomycoides ludwigii* *Zchizosaccharomyces pombe* *Zygosaccharomyces rouxii* *Zygosaccharomyces bailii* *Brettanomyces intermedius* *Zygosaccharomyces lentus* *Candida famata* *Candida guilliermondii* *Candida obutsa* *Candida colleculosa* *Candida kefyr* *Candida krusei*	Induce addition of new thicker membranes that take the shape of their container and heightens the symbiotic effect	Marsh, (2014) Mayser et al., (1995) Steels et al., (2002) Ramadani and Abulreesh, (2010) Şafak et al., (2002) Martínez Leal et al., (2018)

2.1.3 Kombucha and health

2.1.3.1 Nutritional and health benefits of Kombucha

Kombucha has been claimed by Kombucha drinkers all over the world to have many beneficial effects on human health. However, most of the benefits were only studied in experimental models and there is lack of scientific evidence based on human models (Jayabalan et al., 2014).

The health and nutritional benefits of Kombucha to humans are summarized in Table 2.

Table 2: Benefits of Kombucha

SN	Benefits	Source
1	• Detoxifies the blood • Reduces; cholesterol level, atherosclerosis by regeneration of cell walls, blood pressure, inflammatory problems, obesity and regulate appetite, stress and nervous disturbances and insomnia • Alleviates arthritis, rheumatism, and gout symptoms • Promotes liver functions • Normalizes intestinal activity, balance intestinal flora, cure hemorrhoids • Prevents bladder infection and reduce kidney calcification • Stimulates glandular systems • Protects against diabetes and increases body resistance to cancer • Have an antibiotic effect against bacteria, viruses, and yeasts and enhances the immune system and stimulate interferon production • Relieves bronchitis, asthma and headaches • Reduces menstrual disorders and menopausal hot flashes and alcoholic's craving • Improves hair, skin, nail health, eyesight and counteract aging • Enhances general metabolism and more others.	Dufresne and Farnworth, (2000) Afsharmanesh and Sadaghi, (2014) Jayabalan et al., (2008) Bhattacharya et al., (2011) Pauline et al., (2001) Murugesan et al., (2009) Jayabalan et al., (2010) Abshenas et al., (2012) Wang et al., (2014) Dufresne and Farnworth, (2000) Cetojevic-Simin et al., (2008) Ioannides and Yoxall, (2003) Roh et al., (2017) and; Kapp and Sumner, (2019)
2	• An antimicrobial source, for its inhibitory activity on many pathogenic microorganisms like *Agrobacterium tumefaciens, Bacillus cereus, Salmonella choleraesuis, Staphylococcus aureus, Escherichia coli*, *Entamoeba cloacae*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Salmonella enteritidis*, *Shigella sonnei, Staphylococcus epidermis, Leuconostoc monocytogenes, Yersinia enterocolitica*, *Campylobacter jejuni, Helicobacter pylori* and *Vibrio parahaemolytica*	Steinkraus et al., (1996) Greenwalt et al., (1998) Sreeramulu et al., (2000), Sreeramulu et al., (2001) Talawat et al., (2006) Battikh et al., (2012) Malbaša et al., (2011)

2.1.3.2 Health benefits based on *in vitro* and animal studies

The nonhuman subjects literature suggests that Kombucha's health benefits are derived from the tea and the products of fermentation, including glucuronic acid, acetic acid, polyphenols, phenols, and B-complex vitamins, including folic acid (Kapp and Sumner, 2019).

Kombucha drinking is linked with a number of physiological improvements of human. General observation and survey-based reports indicated improvement in general health of population that consumed Kombucha on a regular basis. Such reports were associated with tea consumption practices associated with metabolites present in tea (Huang et al., 2019).

Reports on added benefits of Kombucha beverage are often doubted as an exaggerated hype (Dufresne and Farnworth, 2000;Greenwalt et al., 2000). Considering its long history of global consumption as a health supporting drink, Kombucha is being continuously explored for its inherent properties and validated several facts of general belief associated with it as below;

Anti-inflammatory and anticancer properties (Nasri et al., 2017; Srihari, Karthikesan, et al., 2013). Anti-hypertensive (Lobo et al., 2017), Antidiabetic (Srihari, Karthikesan, et al., 2013; Fu et al., 2014; Aloulou et al., 2012; Kallel et al., 2012), Hepatoprotective (Yarbrough, 2017; Bhattacharya et al., 2011) and Antimicrobial (Sugiharto, 2016; Reygaert, 2014; Siddiqui et al., 2016).

Other studies indicated that, Kombucha extract per kilogram bodyweight fed for 14 days in rats caused significant elevation in plasma and tissue lipid peroxidation (an oxidative reaction) and reduction in delayed hypertensive response, indicative of oxidative stress development (Ram et al., 2000). A study conducted by (Gharib, 2014) where rats were exposed to such high-frequency electromagnetic field (950MHz) for 8weeks, rats fed with Kombucha while being exposed to radiation exhibited reduced response to these adverse effects. This suggested radical-scavenging and ameliorative effect of Kombucha on electromagnetic damage in mammalian tissues. Kombucha adjunct from three oak species were found to possess anti-inflammatory properties when tested in lipopolysaccharide-stimulated human macrophage cells (Vázquez-Cabral et al., 2017).

Production of pro-inflammatory compounds namely nitric oxide, macrophage derived TNF-alpha, and IL-6 was significantly reduced on Kombucha additions (Sun et al., 2015). Additionally, Sun et al., (2015) mentioned that Kombucha fermentation of black tea blended with wheat grass juice can result in markedly better concentration of antioxidant compounds than traditional black tea fermented Kombucha drink.

Enzyme β-glucuronidase is responsible for hydrolyzing glucuronides in the lumen of the gut and generating carcinogenic substances. Brewed Kombucha produces d-saccharic acid-1,4-lactone, a metabolite which inhibits the activity of β-glucuronidase and could prevent colorectal cancer in humans (Wang et al., 2010).

From an early study, Greenwalt et al., (2000) discussed that, additional antimicrobial effect exerted upon both pathogenic Gram positive and Gram negative bacteria by Kombucha after tea fermentation is due to the acetic acid produced during the process. Other researchers noted that, raw material tea itself has the rest of the properties, which are carried over to the Kombucha drink. In contrary, Pure and Pure, (2016) did not find any antibacterial activity in infusions from black tea, banana peel, nettles, and Kombucha made from them. A low concentration of extracts used was opined to be the cause of negative results, which indicated a dose dependency of the material to perform bacterial growth inhibition. However, lemon balm infusion fermented with Kombucha SCOBY resulted in antibacterial activity against eleven (11) wild bacterial species, attributed to acetic acid concentration and other tea components. The SCOBY metabolites having antibacterial properties as reported by (Dutta and Paul, 2019).

2.1.3.4 Adverse health effects associated with Kombucha

Although Kombucha has been reported to have curative effects, there is some evidence of toxicity associated with it. Some individuals have reported dizziness and nausea after consuming certain Kombucha products. Two cases of unexplained severe illness have also been reported following Kombucha consumption (Centers for Disease Control and Prevention (CDC, 1995). Some of the adverse effects related to consumption of Kombucha are summarized in Table 3.

Table 3: Adverse health effects of Kombucha

Adverse health effects	Source
• Lead poisoning and gastrointestinal toxicity in pregnant women and lactating mothers due to the presence of *Anthrax bacillus* in Kombucha fermented in unhygienic conditions	Sadjadi, (1998)
• Side effects like allergic reactions, jaundice, nausea, vomiting, and head and neck pain related to consumption of Kombucha were reported in four patients in HIV-positive patients that consumed Kombucha	Gamundi and Valdivia, (1995)
• Symptomatic lead poisoning requiring chelation therapy by a couple that consumed Kombucha for 6 months, which was brewed in ceramic pot	Phan-Thanh and Montagne, (1998) and Sabouraud et al., (2008)
• Acute renal failure with lactic acidosis and hyperthermia and hyponatremia	SungHee Kole et al., (2009)
• Lactic acidosis	Kapp and Sumner, (2019)
• Toxic hepatitis after consuming Kombucha daily for two years	Nummer, (2013)

• An outbreak of cutaneous anthrax reportedly from applying the Kombucha mushroom to the skin as a painkiller • Metabolic acidosis	Centers for Disease Control et al., (1994)

2.2 Food safety culture and product safety

Food safety in food industries or organizations mainly depends on the food safety culture in the industry or organization in question. Although the concept of food safety culture is still in the developmental stages, multiple researchers have suggested that effective food safety systems and practices need to be shared at all levels not just top management, and communication is an integral part in food safety culture (Yiannas, 2008).

There is no common definition for food safety culture. However, Yiannas (2008) views it as how and what the employees in a company or organization think about food safety because it is the food safety behaviors that they routinely practice and demonstrate.

Griffith et al. (2010) identified six indicators of safety culture that may be applied to food safety as; management systems, leadership, communication, commitment, environment and risk awareness, perception and risk taking behavior. These researchers have gone so far as to include the food safety culture of the organization as a contributing risk factor that can increase the likelihood of a foodborne illness (Griffith et al., 2010). Other researchers feel that studying all facets of the food safety culture within a retail food establishment can lead to improving the compliance of employee-based behavioral practices, which in turn could lead to fewer foodborne illnesses (Griffith et al., 2010). Many perceived barriers have been linked to lack of handwashing and other poor food handling practices in restaurants. Not much information exists on the food safety culture in food industries in Uganda.

2.2.1 Quality and safety aspects of Kombucha

Food safety is defined as the degree of confidence that food will not cause harm or sickness to the consumer when it is prepared, served and eaten according to its intended use (World Health Organization, 2003). Foodborne diseases are an increasing public health problem which are responsible for considerable morbidity and mortality globally (Linscott, 2011). Therefore, safety of food continues to be of great importance to not only consumers but also the entire food industry and regulatory authorities. Increasing cases of consumption of unhealthy food are being reported and approximately 76 million cases of illness, 325000 hospitalizations and 5000 deaths each year are reported in the United States alone (Mead et al., 1999). There are also documented presence of pathogens in traditional fermented foods (Lyumugabe et al., 2010).

In Uganda, for Kombucha to be considered as quality and safe for human consumption it must have passed all parameters as specified in the Kombucha specification (UNBS, 2019), these include physicochemical (alcohol and acetic acid content), heavy metals; (lead, arsenic, mercury and cadmium) and microbiological (*Escherichia coli*, Yeast and molds, *Staphylococcus aureus* and *Salmonella* spp.).

Earlier studies done by Dutta & Paul, (2019) in India also indicated the importance of the above quality and safety parameters in Kombucha. A recent study in Uganda on quality and safety of Kombucha by Galiwango (2021) noted a high total plate count above the recommended maximum levels (5000 to 50000 CFU/ml). This was linked to 80% of the Kombucha preparation premises being unhygienic which encouraged product contamination.

2.3 Knowledge and practices of processors on food safety

Food safety culture relates to the knowledge and practices of processors and consumers. For the purpose of this study, the interest was on Kombucha processors. Prior to this study, there were no known published reports on the knowledge and practices of Kombucha processors in Uganda. However, Byakika et al., (2019), carried out such studies for other products like Obushera. Generally, good food safety knowledge and practice should lead to overall improved product safety. This is because the food handlers know how best to produce food in a safe and hygienic environment that does not compromise the safety and quality of the food.

The same principles and suggestions given by York et al., (2009) on restaurants may be implemented in the Kombucha industries. York et al., (2009) conducted focus group discussions of food service workers and found that time constraints, inadequate training, inconvenience, and not having enough resources were identified as barriers to performing safe food handling practices related to three areas: time/temperature control, personal hygiene, and cross contamination.

In addition, York et al., (2009) suggested that, when a restaurant provided training that focused only on knowledge, employees received no training that would help them to overcome these barriers. Having knowledge of food safety is not a predictor of correct performance of the task, especially with barriers such as time constraints, poor training and lack of resources to overcome.

York et al., (2009) further noted that, when employees working together have the same attitudes and beliefs concerning a practice, there is a better chance of conformity with the standards for that practice. Several studies have reported on the food safety culture in food processing plants but none have investigated its implementation in food service operations. Therefore, the objective of that study was to assess food safety practices contributing to food safety culture in food service operations.

In Uganda, the Food and Drugs Act of 1959 covers food and drug regulations but does not exhaustively include all the food safety requirements. National Food and Drugs Authority (NDA) is the national regulatory body under the Ministry of Health with the responsibility of implementing food policies through its food desk. NDA in conjunction with UNBS ensures the safety and wholesomeness of food for consumers by playing roles including; food manufacturing and processing site inspections, licensing, product registration, monitoring and good hygiene practices training for food handlers.

The safety of food products is determined in part by processors' knowledge, attitudes and practices – KAP as highlighted by (Martini, 2018). Studies in Uganda have reported discrepancies in the link between the food safety KAP of processors and the microbiological quality of foodstuffs (Baluka et al., 2015).

Occurrence of foodborne illnesses is at times attributed to the improper handling of the food items at consumers' homes (Redmond and Griffith, 2003). Mishandling of food can occur during preparation, handling and/or storage of food as noted by Knabel, (1995) and numerous studies

have shown that the mishandling of food occurs because consumers have inadequate knowledge about food handling practices (Altekruse et al., 1996; Knabel,1995; Redmond and Griffith, 2003). Therefore, educating consumers on safe food handling practices can achieve prevention and control of foodborne illnesses (Jevšnik et al., 2008).

Several studies have shown that food safety knowledge and self-reported behavior of food handlers and consumers do not co-relate well (Al-Shabib et al., 2016; Redmond and Griffith, 2003). However, such reported behaviors provide an insight into what consumers know and what they need to be educated about (Redmond and Griffith, 2003). Foodborne diseases can be prevented if control measures and proper food handling and preparation practices are applied (Azevedo et al., 2014; Milton and Mullan, 2010; Powell et al., 2011).

2.4 HACCP

Hazard analysis and critical control point (HACCP) is a preventive approach to control biological, chemical, physical and allergens hazards in food business operations (UNBS, 2017). It is a risk management system, which identifies, evaluates, and controls hazards related to food safety throughout the food supply chain and its implementation is guided by scientific evidence of risks to public health (UNBS, 2017). The standard further states that, HACCP is a quality assurance system to identify, assess and control potential hazards as well as a control system that focuses on prevention.

According to UNBS (2017), Food safety and quality are often subjected to great stress due to efforts to reduce costs and increase efficiency along the food production chain. Oftentimes, food production companies are confronted with undesired compounds found in their products. These substances can be biological, chemical or physical in nature and gain entry into food products at any stage of production. The consequences of their presence in food are incalculable leading to product recalls, with enormous financial losses as a direct consequence; loss of image, not only for the affected products, but also for other products produced by the company as an indirect consequence. Additionally, these undesired compounds can be injurious to consumers and at worst, fatal. This therefore necessitates implementation of food safety systems to ensure supply of food products that are not harmful to consumers (UNBS, 2017).

According to the US 130: 2017, HACCP requirements, a complete and actual description of the pre-requisite programs (PRPs) for the food business shall be availed by the food manufacturers and handlers. The procedures belonging to the PRPs shall be appropriately specified and documented, operational, integrated into the HACCP system, and verified. The PRPs shall comprise good practices, appropriate food legislation and appropriate codes of practices.

The existing good hygiene practices and good manufacturing practices are standard practices that are legally binding guidelines which the food industry can use to ensure food safety (Chekol et al., 2019). These codes are obtained from the Codex general principles of hygiene/international principles and adopted in Uganda by coordination of UNBS. Standards are developed and promoted by UNBS as the Uganda's Secretariat at national, regional and international levels (UNBS Act, 2013).

Food contamination occurs through poor food handling practices, which results in numerous food borne diseases. These diseases are the major causes of morbidity and mortality. Globally, more than 50% of the total food poisoning cases were attributed to improper food handling procedure according to reports by (Najah et al., 2019).

Hazardous pathogens and physical hazards may causes life threatening health problems if they come in contact with food (Najah et al., 2019). Such contaminants get access to contaminate food mainly due to food handler's poor knowledge and negligence during handling activities. Moreover, low financial resources, inadequacy food safety law, in availability of food establishment guidelines and standards, as well as poor monitoring and evaluation systems of food establishments play an important role in poor food handling practices (Najah et al., 2019).

The Uganda standard, (US 130:2017), further details that, HACCP can be applied throughout the food chain from primary production to final consumption. This varies from postharvest activities to restaurants, hotels, schools, hospitals, food processing units/factories/industries as well as pharmaceutical industries. In addition to enhancing food safety, implementation of HACCP can provide other significant benefits such as improving product quality, creating a good reputation and boosting customer confidence, increasing product sales and profit, reducing final product losses due to non-conformances, and enhancing staff morale and loyalty. In addition, the application of HACCP aids inspection and audits by regulatory authorities.

The implementation of HACCP is guided by seven established principles and when a deviation occurs indicating that control has been lost, it is detected and appropriate steps are taken to re-establish control in a timely manner. This ensures that potentially hazardous products do not reach consumers. In addition, an effectively implemented HACCP system is capable of accommodating changes such as advances in equipment design, new information concerning health hazards or risks, and new processing procedures (UNBS, 2017). HACCP emphasizes quality control and food safety, the HACCP approach is used because has become an accepted food safety standard internationally (Wahidah et al., 2012).

HACCP document as a control process has not built yet encouragement of consumers and local government to produce similar quality of product for each production, and becomes a responsibility for SMEs to maintain food safety (Najah et al., 2019).

The HACCP system consists of five preliminary steps; assembling the HACCP team, describing the product, identifying intended use, constructing process flow diagram, and onsite verification of the flow diagram. The system also consist of seven principles of Hazard analysis, determination of critical control points (CCP), establishing critical limits for each CCP, establishing monitoring systems, corrective actions, establishing verification/validation procedures, documentation and record keeping (UNBS, 2017).

HACCP implementation is not a UNBS requirement for product certification. However, it is a requirement for food safety systems certification and assurance that, the food produced is safe for human consumption (UNBS, 2017).

Certification involves the issuance of a certificate or mark (or both) by a third party to demonstrate that a specific product, system or service meets a defined set of requirements such as safety, fitness for use and/or interchangeability characteristics for that product or process usually specified in a standard (UNBS, 2018). According to UNBS, 2018 some of the benefits of certification include; winning consumer confidence in certified products which results into increased market share and consumers' ability to identify the products conforming to quality standards thus making quick decisions in favour of quality products.

Creation of products/systems' better image in both national and international markets resulting into mutual recognition schemes where countries recognize each other's products thus easing entry

into regional and foreign markets. This also enables acceptance and promotion of new products in the markets and safeguarding the image and reputation of the manufacturer.

Protection against unfair competition from inferior products/systems on the market through easy identification of the products that conform with the standards since they carry the quality mark. Government and international bodies also rely on certified products for their purchases as a government requirement for local purchases to be certified.

Provides technical audit of product quality and process control procedures. This means that the manufacturer gets technical advisory services and information at little or no cost that would be obtained at very high cost.

Kombucha HACCP plan development, training and implementation is effective in limiting significant food safety hazards in the products. Aber et al., (2019) noted in the earlier study on HACCP plan development and validation in Uganda done in amaranth vegetable value chain, that HACCP plan implementation is effective in preventing hazards. Similar studies by Gandhi, (2009) on production of soymilk also emphasized the same.

CHAPTER THREE

METHODOLOGY

3.1 Study design

A mixed approach involving three study designs was adopted for this research. Firstly, a descriptive cross-sectional design was used to evaluate the knowledge and practices of Kombucha processors in Uganda. Secondary data on the quality and safety aspects of certified and uncertified Kombucha products were obtained from the UNBS database after experimental study design. A longitudinal and observational study design was used to develop and validate a HACCP plan for Kombucha.

3.2 Study area

This study was conducted in Kampala, Wakiso, Mityana, Mbarara, Ntungamo, Kibale and Kasese districts in Uganda. These districts were considered because they contain most of the SMEs producing Kombucha (**Appendix 3**). The products are mainly sold in Kampala district since it is a major marketing and trading area for processed products.

3.3 Sample size and participants

Initially the sample size was intended to comprise of companies involved in Kombucha processing including those with or without certified products. According to UNBS (2020), there were 25 companies with certified products. The number of companies with uncertified products was not known but was assumed to be equal to that of those with certified products (therefore giving a total of n= 50). Using an online sample calculator (Raosoft Inc, 2004), a margin of error of 5%, confidence level of 95% and an estimated population size of 50, the sample size was estimated as 45 companies. A non-response rate of 10% was considered thus giving a sample size of 50. Thus 25 companies with certified products and 25 with uncertified products were intended for inclusion in the study. It was also planned to interview 50 processors (1 processor per company x 50 companies = 50) to ascertain their knowledge and practices with respect to product safety and quality. The participants targeted were those in positions of either quality assurance manager, quality supervisor, quality controller or anyone directly concerned with production and quality management. These are the key personnel expected to have basic knowledge in food processing

and safety. They also monitor and enforce standard operating procedures, standards and quality assurance in their facilities.

3.3.1 Limitation of the study

At the time of carrying out this study (March 2020 – June 2021) Uganda was in a COVID-19 pandemic lockdown. Most Kombucha processing companies had closed business and very few were willing to receive research students or accept any interviews. Only a total of eight companies (four with certified products and four with uncertified products) were receptive and thus considered for this study. Consequently, only eight (8) participants were interviewed to establish the knowledge and practices of processors.

3.4 Evaluating knowledge and practices of Kombucha processors in Uganda

Face-to face interviews with eight processors (four from companies with certified products and four from companies with uncertified products) were carried out using a researcher-administered questionnaire (**Appendix 4**). The questionnaire used earlier for Obushera was adopted and modified (Byakika et al., 2019).

The questionnaire was composed of sections to capture information on; company profile, processing of Kombucha, knowledge of basic food safety and hygiene aspects, knowledge of relevant standards/specifications essential for beverage production, execution of appropriate or recommended practices which were verified by researcher, HACCP system, Good Hygiene Practices (GHP), Good Manufacturing Practices (GMP) and product certification among others. Visual observations for certain practices such as disinfecting of shoes prior to entry in the facility, use of protective clothing, hand washing/sanitization facilities, documentation and record keeping was also done. The questionnaire had provisions for ‘’YES’’ or ‘’NO’’ responses with respect to knowledge and practices questions. Companies with uncertified products were asked to indicate their willingness to obtain relevant knowledge and comply with the relevant standards and quality management systems during Kombucha production.

3.5 Evaluating the quality and safety of Kombucha on the Ugandan market

Data on quality and safety of Kombucha products from the eight (8) participating companies were obtained from the UNBS testing laboratory database. The data obtained was for samples analysed between 2019 to 2020. The data set comprised of the sample identifier, company name, product name and results of analyses based on the Kombucha specification (US 2037:2019). The parameters tested included; alcohol content, acidity (as acetic acid), microbial counts (yeast and molds, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp), and heavy metals namely; lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (Ar).

3.6 Developing and validating a Hazard Analysis and Critical Control Point plan for Kombucha processing

One willing company with uncertified Kombucha product was selected for purposes of developing and validating a HACCP plan for Kombucha. A detailed recommended HACCP plan was developed following UNBS (2017) and UNBS ISO (2015). A summary of HACCP plan development guidelines is shown in **Appendix 5**. The HACCP plan was developed and given to the company for adoption and implementation. The Company employees were trained in HACCP system implementation using Uganda standard for HACCP requirements (US 130:2017). Four employees were trained and these included; Marketing manager, Factory supervisor, Quality controller and Managing Director. The interested company is located in Mbarara district, Kakoba sub-county with a branch in Isigiro district and this company has been in operation for four years with a production capacity of 5000 L per day.

Monitoring of HACCP plan implementation was done through evaluating record keeping and documentation, onsite observations and product testing for compliance with product standard. HACCP plan validation was done practically by in-plant observation of production processes as stipulated in UNBS (2017) and UNBS ISO (2015). Performing tests of the final product and those in transition/process was done for microbial and physicochemical properties. A baseline to assess product quality and documentation processes was carried out prior to adoption of the proposed HACCP plan. Post adoption tests were done for one month at intervals of one week to analyse for microbial counts, alcohol and acetic acid content and heavy metals as specified in (UNBS, 2019). Two samples were obtained at each sampling point and products observation for colour, odour and taste change was done during one-month period. Inquiries on HACCP implementation challenges

were highlighted by the company among which included; Unwillingness of some workers to observe HACCP perquisite programs, high costs of products routine testing on product specifications and failed observation of some CCPs during production.

3.7 Analyses

3.7.1 Physicochemical analyses

Alcohol content was determined by the specific gravimetric method (UNBS, 2014). Titratable acidity (TA) as acetic acid was obtained by titrating 10 ml of the sample against a standardized solution of 0.1M NaOH with phenolphthalein as the indicator (AOAC, 1990). Acidity was computed using an equation below.

$$\text{Acidity (\% acetic acid)} = (0.1M\ NaOH \times volume\ of\ NaOH\ (L) \times MW)$$

Where; MW = 60.05

3.7.2 Microbiological analyses

Ten-fold serial dilutions of Kombucha samples were prepared using ¼ strength Ringer's solution (Oxoid Ltd, Basingston Hampshire, England). *E. coli* counts were determined by pour plating selected serial dilutions of the sample in *E. coli*-coliforms chromogenic agar (Laboratorios CONDA, Madrid, Spain) and plates incubated at 37 °C for 24 h (UNBS, 2008). Yeasts and molds were enumerated by spread plating on Potato Dextrose Agar (Pronadisa Laboratories Conda S.A Madrid Spain) followed by incubation at 30°C for 5 days (UNBS, 2012). *Staphylococcus* spp. Such as *Staphylococcus aureus* counts were determined by pour plating on Baird Parker Agar (BPA) (Laboratorios, CONDA, Madrid, Spain) containing tellurite egg yolk supplement (Laboratorios, CONDA, Madrid, Spain) followed by incubating at 30 °C for 48 h (UNBS, 2014). Detection of *Salmonella* spp. was done according to UNBS (2017). For this Buffered Peptone Water (Laboratorios, CONDA, Madrid, Spain) was used for pre-enrichment of the sample, followed by incubating at 37 °C for 24 h. This was followed by a second enrichment step in Rappaport Vassiliadis Soy broth (Laboratorios, CONDA, Madrid, Spain) and incubation at 41.5 °C for 24 h for *Salmonella* spp. Streaking to determine the presence of *Salmonella* spp. was done on Xylose Lysine Deoxycholate agar (Laboratorios, CONDA, Madrid, Spain) and Brilliant Green Agar (Laboratorios, CONDA, Madrid, Spain) followed by incubating the plates at 37 °C for 24 h.

Presumptive *Salmonella* spp colonies were confirmed by streaking on Triple Sugar Iron (Laboratorios, CONDA, Madrid, Spain) and Simmon's Citrate agar (Laboratorios, CONDA, Madrid, Spain) slants and incubating at 37 °C for 24 h.

3.7.3 Heavy metal determination

The determination of heavy metals was based on the analyses of the ash obtained by dry ashing at 400 °C (UNBS, 2007). Lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (Ar) were determined using inductively coupled plasma optical emission spectrometry.

3.8 Data analysis

Descriptive statistics was used to compile data on the knowledge and practices of Kombucha processors. A mark/point was scored for each correct response while no point was given for a wrong response for data on knowledge and practices of processors. Total points per processor per section were computed as a percentage. Final percentage scores per section were categorized as; 0–25% (very poor), 25–50% (fairly poor), 50–75 (fairly good) and 75–100% (very good). A Chi-Square test ($\alpha = 0.05$) was used to determine associations between Kombucha safety and quality with food safety knowledge and practices of processors. Results of analysis of Kombucha (total titratable acidity, alcohol content, microbial counts and heavy metal analysis) were checked for conformation with the Kombucha specification. Means of data on samples tested before and after HACCP plan development and implementation were compared using a t-test. The significance level was set at < 0.05. All data were analyzed using Statistical Package for Social Science (SPSS), version 19.0.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Knowledge and practices of Kombucha processors in Uganda

4.1.1 Characteristics of the Kombucha processors

Table 4 summarizes the major characteristics of the Kombucha processors (n = 8) interviewed. The estimated samples size of 50 was not achieved because a number of companies ceased operations during the COVID 19 pandemic lockdown. Some companies were also unwilling to participate in the study. Despite the low number of respondents in this study, the respondents were relatively representative of Kombucha processors in Uganda considering that their factories were located in different districts and that several of the companies had also closed business. There was an equal proportion of processors with certified and uncertified products. Most of the processors (n=6) had 2-4 years' experience in Kombucha production. This can be explained by the fact that, commercial Kombucha processing is relatively new in Uganda having started in July 2019 with first sample certified by UNBS (cims.unbs.go.ug as of October 2021).

A high proportion of the processors (n=6) had very good knowledge and practices related to food safety. A number of studies on knowledge and practices of processors of fermented products have reported a high proportion of processors with very good knowledge and self-reported practices related to food safety (Mukisa et al., 2020; Byakika et al., 2019; Muwanguzi, 2018; Kiberinka, 2018; Akabanda et al., 2017). High scores on knowledge and practices of processors may translate into improved product quality and safety (UNBS, 2017). However, some studies have reported that high scores on knowledge and practices may not necessarily translate into products conforming to standards (Byakika et al., 2019; Akabanda et al., 2017). This is because the operators knew what the standard requires but practical implementation in the industry is hardly practiced.

Table 4: Characteristics of the Kombucha processors interviewed in the study

Characteristic	Frequency (n = 8)
Certification status	
Uncertified	4
Certified	4
Location (district)	
Kampala	2
Wakiso	1
Mityana	1
Mbarara	1
Ntungamo	1
Kibale	1
Kasese	1
Kombucha processing experience (years)	
< 2	2
2-4	6
Food safety knowledge Category	
Very Good	6
Fairly Good	2
Food Safety Practices Category	
Very Good	6
Fairly Good	1
Fairly Poor	1

4.1.2 Knowledge of Kombucha processors

Table 5 summarizes the food safety knowledge of the Kombucha processors interviewed. This table shows the frequency of correct and wrong responses to the food safety knowledge questions posed to the processors. Although the processors had fairly good to very good knowledge scores (Table 4), some were ignorant about key food safety issues. All processors (n = 8) were knowledgeable about the importance of product certification, hand washing practices, use of clean raw materials and that eating and drinking in the processing area can lead to product contamination. Food product certification is a reflection of standards implementation and uptake thus correlates with food safety improvement (Teixeira and Sampaio, 2013). Food safety certification does not only provide proof that the product itself is safe to use but also warrants that the business holding this credential has met both the professional and ethical standards to run a business selling food to the public (Kaczorowska et al., 2021). Additionally, personnel hygiene through hand washing and cleanliness is important in prevention of food product contamination (Djekic et al., 2014; Margas and Holah, 2014).

Most processors (n = 6) did not know the importance of sanitizing utensils. Half of the respondents (n = 4) had no knowledge about the prerequisites of HACCP (i.e GMP/GHP) and a HACCP system. HACCP system implementation is key in identification of food safety hazards and preventing them before they can cause significant food safety risks to end-product consumers (Liu et al., 2021). Furthermore, the HACCP system and approaches facilitate trade at national, regional and international level as a number of countries adopt similar standard practices in ensuring control of food safety hazards which lead to food borne illnesses as reported by (Caswell and Hooker, 1996).

However, HACCP system implementation is guided by initially complying with the HACCP prerequisites for example GMP and GHP (UNBS, 2017). These prerequisites ensure that food handlers and the environment are safe for hygienic and safe food production (Roberts and Sneed, 2003). Conversely, failure to observe the prerequisite programs may lead to retained challenges in HACCP plan implementation (Baş et al., 2006). HACCP plan implementation should be applied in all stages of food chain production to ensure that safety of the final product is not compromised (Pierson, 2012).

Table 5: Food safety knowledge of Kombucha processors

Food safety knowledge questions/statements	Response (n)	
	Correct	**Wrong**
Necessary to have your product certified by UNBS	8	0
Hand washing prior to processing contributes to Kombucha safety	8	0
Hand washing after touching money is important for Kombucha safety	8	0
Hand washing after using washrooms is important for Kombucha safety	8	0
Hand washing after touching the body is important for Kombucha safety	7	1
Hand washing after using the phone is important for Kombucha safety	6	2
Hand washing after each break is important for Kombucha safety	7	1
Hand washing after handling garbage is important for Kombucha safety	8	0
Sanitizing utensils increases the risk of Kombucha contamination	2	6
Washing utensils with detergent makes them sterile	8	0
Eating and drinking during processing increases the risk of Kombucha contamination	8	0
Diarrhea, vomiting and stomach pain arise from drinking Kombucha made unhygienically	5	3
Microorganisms are found on the skin, hair and hands of processors and they are potential disease causers if they get into Kombucha	6	2
The use of clean and well stored raw materials is vital for Kombucha safety	8	0
Pathogens change the sensory properties of Kombucha	7	1
Monitoring of water quality is important in ensuring Kombucha safety	7	1
What is GMP/GHP program?	4	4
What is a HACCP plan?	4	4
What do you understand by a product standard/ specification?	5	3
Does Uganda have a product specification for Kombucha?	8	0
Name the standard/specification for Kombucha?	4	4

4.1.3 Food safety practices of Kombucha processors

Table 6 summarizes the self-reported food safety practices of the Kombucha processors interviewed. This table shows the frequency of correct and wrong responses to the food safety practice questions posed to the processors. All the processors (n = 8) claimed to have good hygiene practices, sanitize utensils, had vermin proof storage facilities or used treated water for processing. Food processors are expected to observe proper hygiene and sanitation methods as the chances of food contamination largely depend on their health status and hygiene practices. All of the processors (n = 8) indicated that, they usually washed their hands before handling food and after handling money or any contaminated surfaces. Effective hand washing, therefore, is an essential control measure for prevention of pathogens (Ifeadike et al., 2014). In earlier studies by Rossoni and Gaylarde, (2000) sodium hypochlorite was mentioned to sanitize equipment by killing or removing adherent cells of microorganisms like *Escherichia coli* and *Staphylococcus aureus.* Its application during cleaning is hence relevant in sanitizing equipment before Kombucha production thus ensuring product safety (Rossoni and Gaylarde, 2000). Food industries must use portable water that meets microbiological, physicochemical and organoleptic characteristics as indicated by national standards (UNBS, 2014). Water when used as a processing aid has direct quality impact on the final product quality, therefore clean and safe water must be used in Kombucha production (Brennan and Grandison, 2012). Water management is critical in the food sector, both in terms of water quality and quantity. This is because if not adequately treated, reused water might contaminate the finished product compromising its safety. (Kirby et al., 2003).

About half of the processors (n = 4) did not use: (i) objective methods for testing product quality, (ii) running water for washing bottles and their caps, and (iii) did not have the Kombucha specification and a HACCP plan. These results were similar to those in earlier studies on fermented traditional foods like Obushera and beef (Jeffer et al., 2021; Byakika et al., 2019; Baluka et al., 2015). All of these findings indicated that the food chain's food safety performance was poor, owing to poor sanitation, hygiene, and handling standards, as well as inadequate HACCP plan implementation. Therefore, HACCP-based training and robust preventive, intervention, and monitoring systems should be strengthened in food production in Uganda.

Table 6: Self-reported food safety practices of Kombucha processors

Food safety practice questions/statements	Response (n)	
	Correct	Wrong
Have a foot bath at the entry to the facility	7	1
Check the length and cleanliness of the nails of the processors	8	0
Ensure workers wear proper head gear during processing	8	0
Ensure workers wear closed shoes during processing	8	0
Processors remove jewelry and other accessories before processing	8	0
Ensure workers wear separate clothes specific for processing	8	0
Processors are examined for contagious diseases	7	1
Workers wash and sanitize their hands before and during work	8	0
Sanitize of utensils before processing	7	1
Sanitize of utensils after processing	8	0
Sanitize packaging material before use	5	3
Facility is vermin proof storage	8	0
Use treated water for Kombucha processing	8	0
Use of objective methods to test readiness of Kombucha	4	4
Adequately clean packaging materials (use soap, clean water and sanitizer)	7	1
Use running water/regularly change water for washing used bottles and cups	4	4
Wash utensils after Kombucha processing	8	0
Store utensils in a clean area separate from raw materials	5	3
Use Kombucha preparation utensils for other purposes	5	3
Dispose garbage in a covered garbage receptacle	5	3
Have /follow a Kombucha specification, if yes, state it	4	4
Follow a Hazard Analytical Critical Control Point (HACCP) plan	3	5

4.2 Quality and safety of Kombucha on the market in Uganda

Table 7 summarizes conformity assessment of Kombucha from different products and their conformance with specifications. All the samples (n = 8) evaluated passed the specifications for acetic acid, heavy metals, *Staphylococcus aureus*, *E.coli* and *Salmonella* spp. However, only (n = 4) and (n = 5) of the samples passed yeast and molds and alcohol content respectively. This resulted into only (n =2) overall conformance of the products with the Kombucha specification. It is a requirement by UNBS that for a product to be certified as safe for final consumption it must comply with all the requirements in the product specification (UNBS, 2018). Therefore, some samples conforming partly to the requirements in the standard did not guarantee their product safety thus the high rate in overall non-conformance with the specification.

Poor chilling methods along the product value chain may be the main explanation for (n = 4) failure on the yeast and molds parameter for both certified and uncertified Kombucha products. It is important to note that, some Kombucha products might not be have been pasteurized during production. This could have allowed the leftover yeasts from the SCOBY to continue growing resulting in production of high levels of alcohol and other bi-products in the Kombucha while it was stored at room temperature (Varzakas, 2020). The results of this study in terms of food safety are similar to a study done in Rwanda's SMEs producing pineapples (Mukantwali, 2014). Detailed analyzed data is shown in **Appendix 6**.

A chi square test was used to determine the relationships between certification status, product food safety conformance data and knowledge and practice levels but none was detected ($p>0.05$). This could be due to the small sample size used in this study (McHugh, 2013; Lewis and Burke, 1949). This is explained by the minimum recommended sample size for chi square test requirement as anticipated to have an expected value above five (5) for each category (Ugoni and Walker, 1995; Roscoe and Byars, 1971). Detailed Tables 13, 14, 15 and 16 of the association are in **Appendix 7** and raw data tables shown in **Appendix 8**.

Table 7: Conformity assessment of Kombucha products and their conformance with specifications

	Specification requirements (US 2037:2019)										
Product	**Staph (CFU/ml)**	***E. coli* (MPN/ml)**	***Salmonella* spp (25ml)**	**Yeast (CFU/ml)**	**Alco (%v/v)**	**Acidity (g/L)**	**Lead (mg/L)**	**Arsenic (mg/l)**	**Mercury (mg/l)**	**Cadmium (mg/l)**	**Overall conformance**
*C1[+]	P	P	P	P	P	P	P	P	P	P	P
C2	P	P	P	P	F	P	P	P	P	P	F
C3	P	P	P	F	P	P	P	P	P	P	F
C4	P	P	P	F	P	P	P	P	P	P	F
*UC1	P	P	P	P	F	P	P	P	P	P	F
UC2[+]	P	P	P	P	P	P	P	P	P	P	P
*UC3	P	P	P	F	F	P	P	P	P	P	F
UC4	P	P	P	F	P	P	P	P	P	P	F
STD	Absent	Absent	Absent in 25 ml	100 Max. (CFU/ml).	0.5 (Max non-alco) 0.6-15 (Alco)	2 Max.	0.05 Max.	0.05 Max.	0.001 Max.	0.003 Max.	
(n) Conformance	8	8	8	4	5	8	8	8	8	8	
(n) Non-conformance	0	0	0	4	3	0	0	0	0	0	

*STD = Standard, C = certified products, UC = Uncertified product, Staph= Staphylococcus aureus, Max = Maximum, Alco = Alcohol, non-alco = Nonalcoholic, P = Passed, F = Failed. *product was labelled as non-alcoholic.* [+] A sample is considered to have passed or met overall conformance if it met all the parameters in the specification.

4.3 Hazard Analysis and Critical Control Point plan for Kombucha processing

Technical staff members (n=4) of one company with uncertified Kombucha product were trained in HACCP system following HACCP plan guidelines as explained in HACCP requirements (UNBS, 2017; UNBS ISO, 2015). After the training a process flow diagram (Figure 2) was developed. Prior to this, the company had not developed a process flow diagram. Each step on the flow diagram was assessed for potential hazards, used to identify critical control points on the HACCP plan. This was important in eliminating the significant food safety hazards (UNBS, 2017; UNBS ISO, 2015; Corlett, 1998).

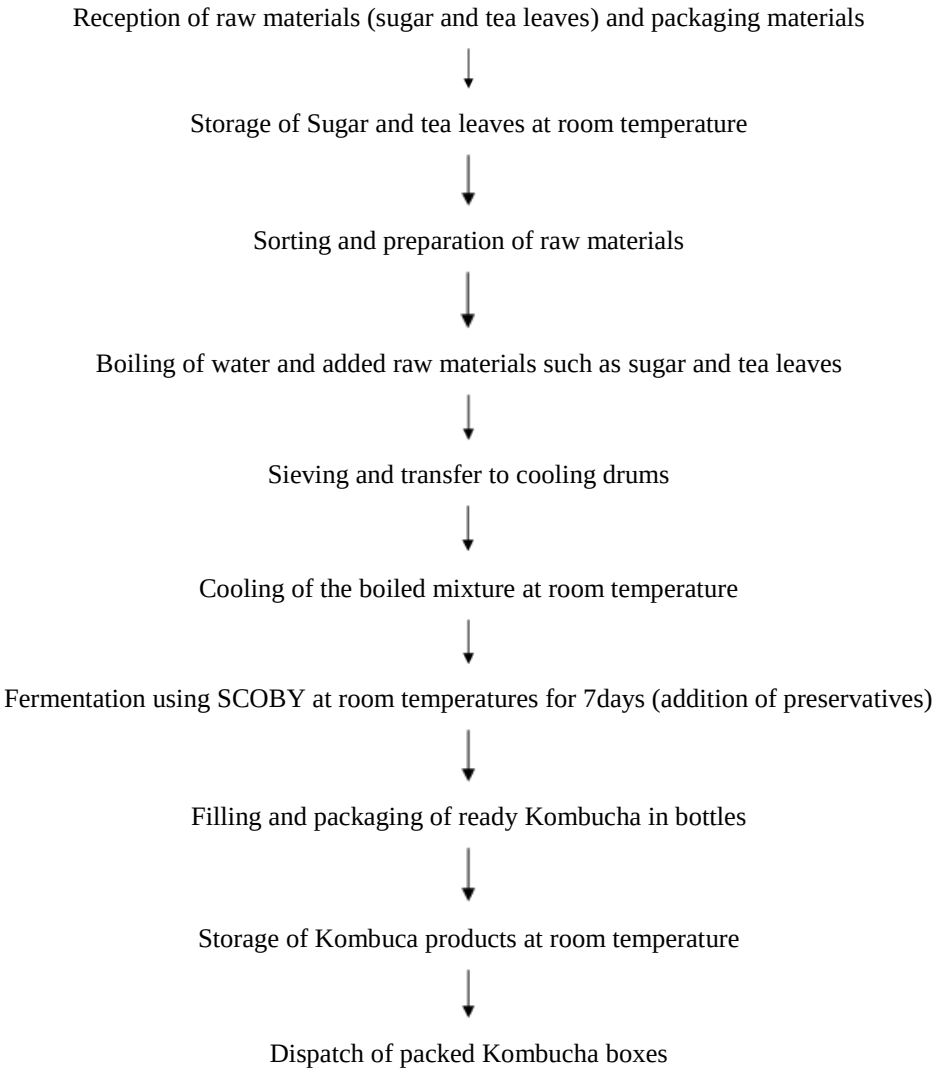

Figure 2: Kombucha flow diagram prior to HACCP plan adoption

A HACCP plan of the company product was developed (Table 8). Three CCPs and five CPs were identified, the CCPs included; Boiling, sieving and pasteurization of the Kombucha. The CPs included; reception of raw materials and other materials, storage of raw materials, fermentation process, packaging and storage of finished Kombucha products. CCPs are important in complete elimination of significate food safety hazards or reduced them to acceptable levels that does not compromise consumer safety and health (UNBS, 2017; UNBS ISO, 2015; Corlett, 1998). At these CCPs, critical limits were established as criterion for separating acceptability from unacceptability for examples maximum limits for pathogenic microorganisms as detailed in the Kombucha specification (UNBS, 2019). At each CCP particular control measures like time-temperature regimes during boiling were established and monitored to prevent any deviations from the critical limits. This is because loss of control at a CCP would lead to failure in eliminating specified food safety hazards hence affecting safety of the final product (UNBS ISO, 2015; Corlett, 1998). The HACCP plan was thereafter, given to the company for adoption. Training photos are in **Appendix 10**.

Table 8: The HACCP control chart for Kombucha developed in this study

Process Step: CP or CCP#	Hazard	Critical Limits	Monitoring Procedure	Frequency	Preventative Measure	Corrective Action	Record	Responsible Person
Reception of raw materials (sugar, tea leaves) and packaging materials **CP# 1**	• Biological hazards such as insects from tea leaves • Chemical hazards such as migratory materials from plastic packaging materials • Physical hazards (small stones and dust from sugar or tea leaves)	No unqualified product to be used	Apply supply quality assurance by use of standards	Each supply	• Qualified raw materials and ingredients • Checked MSDs • Approved suppliers list • Use of specifications	• Change of suppliers or brand • Employees training	Material receiving report	Assigned Quality assurance Officer
Storage of Sugar and tea leaves at room temperature **CP# 2**	Microorganisms (Yeast and molds)	Not more than 100 CFU/ml	• Temperature log is properly running and monitored • Apply supply quality assurance by use of standards	Routinely (morning and evening)	Proper storage temperature and time in dry place	Reject the raw material	Temperature log sheet: Discard register	Assigned Quality assurance Officer
Boiling water and added raw materials such as sugar and tea leaves **CCP# 1**	Microorganisms (Yeast and molds, *E. coli*, *Staphylococcus aureus* and *Salmonella* spp)	• Not more than 100 CFU/ml for yeasts and molds • Absent in 25ml for *Salmonella* spp • Absent for *Staphylococcus aureus* • Absent for E.coli	Check the CT and time	Each batch	• Heating to boiling point of 100 ◦C • Check the core temperature (CT) of the product • keep records	Adjust the temperature and time by setting the equipment well; Call the engineer to repair	Time and CT log: Maintenance register	Assigned Quality Assurance Officer

Sieving **CCP# 2**	Physical contaminants (insects, small stones and dust	No physical foreign matter	Check the sieve clothes for the right sizes prior to sieving of the product	Each Batch	Prior check of sieve clothes for hygiene and right sieve sizes	Changing the sieve clothes to replace right ones,	Inspection report	Production Manager
Fermentation using SCOBY **CP# 3**	Pathogenic microorganisms and yeasts and molds	No pathogenic microorganism	Monitoring the fermentation conditions like temperature and pH	Each batch	Route checking of the Time-temperature and pH	Adjustment of the temperatures and pH	Time, pH meter and CT log: Maintenance register	Assigned Quality Assurance Officer
Pasteurization of Kombucha **CCP# 3**	Residual Yeast and molds from the SCOBY during fermentation process	Not more than 100 CFU/ml for yeasts and molds	Check the core temperature (CT) of the product and holding time	Each batch	• Heating to boiling point of 85 ◦C and holding for 10 minutes • Check the CT and time • Keep records	Adjust the temperature and time by setting the equipment well; Call the engineer to repair	Time and CT log: Maintenance register	Assigned Quality Assurance Officer
Packaging **CP# 4**	Chemical hazards such as migratory materials from plastic packaging materials	No unqualified product to be used	Visual inspection for foreign materials, hygiene, leaking and following of packaging specifications	Each Pack	• Disinfection of packaging bottles • Personal hygiene and physical inspection • Use of specifications for packaging materials	Retain, rework or discard based foreign material identified	Inspection report	Packaging operator and Quality Assurance Manager
Storage and distribution of Kombucha **CP# 5**	Microorganisms (Yeast and molds)	Not more than 100 CFU/ml	Check the time and temperature regime	Routinely	• Keeping the products at < 4 ◦C for a shelf life 14 days • Check the storage temperature and shelf life and record keeping	Retain or reject based on the product testing panelist	Temperature log: Delivery report	Quality assurance Manger

The HACCP plan was validated by testing samples prior and post HACCP plan implementation. Results of validation are shown in Table 9. Prior to HACCP plan adoption, the products did not meet the yeasts and molds requirement. This might have been due to continued growth of residual yeasts and molds from the added SCOBY during the Kombucha production. The yeasts and molds metabolized sugars to produce alcohol and carbon dioxide (Mukisa et al., 2017). Adoption of the HACCP plan resulted in the products meeting the yeast and molds requirements. This was due to introduction of pasteurization step as a new CCP during process improvement thus resulting in removal of yeasts and molds (Byaruhanga, and Ndifuna, 2002). After HACCP plan adoption, the alcohol content reduced and the acidity increased. The alcohol content after HACCP plan adoption reduced due to post process elimination of yeasts and mold that were responsible for its synthesis. There was no continued fermentation to produce alcohol after yeasts removal during pasteurization (Byaruhanga, and Ndifuna, 2002). The termination of the continued fermentation process led to moderation of the alcohol and acid content, which would ultimately result in improved product shelf life (Gimbi et al., 1997). Other added benefits of moderation of alcohol and acid content in the product included; improved product sensory acceptability, product safety and quality (Farag et al., 2020; Mukisa et al., 2012; Byaruhanga, and Ndifuna, 2002) and reduced incidence of acidosis upon consumption of Kombucha (Farag et al., 2020).

Table 9: Comparison of microbial and physicochemical parameters of Kombucha products pre and post HACCP implementation

Parameter	Before HACCP		After HACCP	
	Sample	Values	Samples (week)	Values
Microbial				
Escherichia coli (CFU/ml)	1	<1	1	<1
	2	<1	2	<1
			3	<1
			4	<1
Yeast and molds (CFU/ml)	1	TNTC	1	<1
	2	TNTC	2	<1
			3	<1
			4	<1
Staphylococcus aureus (CFU/ml)	1	<1	1	<1
	2	<1	2	<1
			3	<1
			4	<1
Salmonella spp. (/25ml)	1	ND	1	ND
	2	ND	2	ND
			3	ND
			4	ND
Physicochemical				
Alcohol content (%v/v)	1	2.1	1	1.1
	2	2	2	1.0
			3	1.1
			4	1.0
	Mean	2.0250[a]		1.0750[b]
Acidity as (acetic acid, g/L)	1	0.5	1	0.9
	2	0.5	2	0.9
			3	0.9
			4	0.9
	Mean	0.5000[a]		0.9000[b]
Lead (mg/L)	1	<0.05	1	<0.05
	2	<0.05	2	<0.05
			3	<0.05
			4	<0.05
Cadmium (mg/L)	1	<0.002	1	<0.002
	2	<0.002	2	<0.002
			3	<0.002
			4	<0.002
Arsenic (mg/L)	1	<0.05	1	<0.05
	2	<0.05	2	<0.05
			3	<0.05
			4	<0.05
Mercury (mg/L)	1	<0.001	1	<0.001
	2	<0.001	2	<0.001
			3	<0.001
			4	<0.001

N = 2 (2 samples per week before HACCP plan adoption). N = 4 (2 samples per week after HACCP plan adoption). TNTC= Too numerous to count, (Dilution factor for E.coli and yeast and molds were $1*10^0$ and $1*10^1$ respectively). ND = Not detected. Means with different superscripts (a,b) in row are significantly different (P< 0.05).

Results for post HACCP adoption showed improved compliance with the Kombucha specification, this implied that the HACCP plan had significant improvement on the quality and safety of Kombucha as in earlier reports (Liu, 2021; Bai et al., 2007). The overall non-compliance of the products before HACCP plan adoption and overall compliance of the products after HACCP plan adoption might have been due to acquired knowledge and skills imparted in participants during the HACCP training (Ghafar et al., 2015; Chang et al., 2003). The HACCP plan training and adoption might have improved the industry's food safety system hence leading to products complying with the specification. Validation process photos are shown in **Appendix 11**.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

1. Kombucha processors (with certified and uncertified products) interviewed in this study had good knowledge and practice with respect to food safety and hygiene.
2. Despite the good knowledge and practices, only a few (n=2) products met quality and safety specifications for Kombucha. This was due to failure in complying with the requirements for yeasts and molds as well as alcohol content.

   Note: The Ugandan specification for Kombucha has a limit for yeasts and molds, yeasts such as *Saccharomyces cerevisiae* are part of the normal flora of the SCOBY and remain after fermentation. Therefore, the presence of yeasts does not necessarily amount to a microbial hazard. Their presence is only likely to lead to production of high amounts of ethanol and early product spoilage. Therefore, need to take this into consideration when revising the maximum limits for yeasts and molds requirement. A company may have to introduce a pasteurization stage after fermentation process to inactivate the remaining flora from the SCOBY just to ensure product stability.
3. HACCP plan development, training and adoption indicated the role of continuous food safety improvement through compliance with Kombucha specification.

5.2 Recommendations

The following are some of the possible areas for further study:

- Need for a detailed study covering a larger number of companies and products over a longer period.
- Need to develop and validate a HACCP plan for SMEs in the production of Kombucha for other companies

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APPENDICES

Appendix 1: Research budget

ITEM	QUALITY	UNIT COST (UGX)	Amount (UGX)
GYC agar	500g (2 tins)	495,000	990,000
VRBL agar	500g	295,000	295,000
Potato Dextrose agar	500g	285,000	285,000
Ringer's tablets	100 tablets/pk	140,000	140,000
Ethanol Absolute	2.5L	175,000	175,000
Cotton wool	500g (2)	10,000	20,000
Sodium hydroxide pellets	500g (3)	30,000	150,000
E. coli-coliforms chromogenic agar	500g	2,500,000	2,500,000
Baird Parker Agar	500g (2 tins)	435,000	870,000
Egg yolk tellurite emulsion	100ml	195,000	195,000
Buffered Peptone Water	500g	295,000	295,000
Rappaport Vassiliadis Soy broth	500g	350,000	350,000
Xylose Lysine Deoxycholate agar	500g	350,000	350,000
Brilliant Green Agar	500g	315,000	315,000
Simmon's Citrate agar	500g	330,000	330,000
Triple Sugar Iron	500g	275,000	275,000
Transport facilitation for field (Industry)	2month	2,500,000	2,500,000
Total			**10,035,000/=**

Source: Taifa Laboratory Supplies

Appendix 2: Work plan for my research in 2020 to 2021

MONTH	WEEK	ACTIVITIES TO BE DONE
July, September and October	Week 1	Proposal development
	Week 2	Proposal development
	Week 3	Objective one; Field work; Obtaining data on Kombucha processors' knowledge and practices from SMEs
October and November	Week 2	Objective one; Fieldwork
	Week 3	Objective one; Field work
	Week 4	Objective one; Field work
November and December	Week 1,2,3,4	Objective two; Kombucha quality and safety laboratory tests on the different Kombucha products
January and February	Week 1,2 and 3	Objective three; HACCP plan development and validation with Kombucha SMEs
	Week 4	Data Analysis
March	Week 1,2,3,4	Report writing
March and May	Week 1,2,3,4	Report writing

Appendix 3: List of Kombucha products certified by UNBS

Small and Medium Enterprises/ Industries producing Kombucha drink in Uganda (Product certified according to UNBS: 2019)

S/N	Company	Product brand
1	Murimo quality millers limited	Bulamu masters alcoholic Kombucha drink
2	Akandi beverages (U) limited	1. Ab akahdi 1 non-alcoholic Kombucha drink 2. Ab akahdi 2non-alcoholic Kombucha drink 3. Ab akahdi 3 non-alcoholic Kombucha drink
3	Mana yera company	Ntugasaze drink - alcoholic kombucha
4	Kombucha products limited	Dr. Kombucha
5	Tayebwa health products limited	Tayebwa Kombucha saana
6	High protenza limited	Protenza detox non-alcoholic Kombucha drink
7	Sawa sawa products (u) limited	Sawa sawa sana
8	Kituzi farm company limited	Kituzi drink, non-alcoholic kombucha
9	Entare beverages	1) Entare sana 2) Entare drink
10	Chaz trading international limited	1) Chaz kiaz kombucha 2) Chaz magic kituzi
11	Besi (u) limited	Kirungi furaha non-alcoholic Kombucha drink
12	Sweet - sana beverages ltd	1) Sweetsana extra - alcoholic Kombucha drink 2) Sweetsana super - alcoholic Kombucha drink
13	Nutricom food and beverages	Jajja - alcoholic Kombucha drink
14	Khaupe beverages and contractors limited	Mzuri kwa afya - non-alcoholic Kombucha drink
15	Mityana foods and beverages limited	Anko non-alcoholic Kombucha drink
16	Kabu Kombucha enterprises (u) limited	Akarusho non-alcoholic Kombucha drink
17	May hawk -smc- limited	Kyeeza alcoholic kombucha
18	Hariss international limited	Riham mugaso non-alcoholic kombucha
19	Mubende foods & beverages limited	Boby alcoholic Kombucha drink

20	Kasese distillers limited	1) Tubonge – non-alcoholic Kombucha drink 2) Kijaana sana – non-alcoholic Kombucha drink 3) Dream – non-alcoholic Kombucha drink
21	Tendo bakery limited - Kampala branch	1) Embogo - alcoholic Kombucha drink 2) Darasa ekituzi - alcoholic Kombucha drink
22	J & d beverages	Karaso - alcoholic Kombucha drink
23	Fredanet trading co limited	Muhumuza alcoholic Kombucha drink
24	Mamma Mia bakery limited	1) Koona Kombucha with GINGER- Non Alcoholic Kombucha Drink 2) Koona Kombucha with MULONDO- Non Alcoholic Kombucha Drink 3) Koona Kombucha KITUZI -Non Alcoholic Kombucha Drink
25	Thaddy investments limited	1) Tayali ginger non alcoholic Kombucha drink 2) Tayali non alcoholic Kombucha drink

Source: https://cims.unbs.go.ug/api/website/

Appendix 4: A questionnaire for evaluating the knowledge and practices of Kombucha processors in Uganda's SMEs

Company name…….. Date……

Location……Product brand (s)……..

Address ……..

Introduction

I am **Rehema Meeme**, a graduate student of MSc. Food Safety and Quality Management at the School of Food Technology, Nutrition and Bioengineering, Makerere University. I am conducting a study on Kombucha production focusing on its safety, quality and compliance with relevant standards. You will be asked questions about the company, knowledge on processing and safety of Kombucha and on practices related to food safety. I will also at the end request for a sample of your product. The results of this study will be used in supporting Kombucha processors to develop and implement a HACCP plan in order to improve the safety and quality of their products.

Request for consent

Before proceeding with this study, I would like to request for your permission to participate in this study. Your identity and that of your products will be confidential and will not appear in any report or published work. If you accept to participate in this study please indicate this by providing your details below and signing

Name of respondent: …….. Signature ……..

Level of Education and Qualification: ……..... Position: ……

Time (years) spent in the Company: ……..…… Date: …….....

Instructions

Please answer the questions honestly. Feel free, where possible, to share proof to support your answers. This could be in form of a document/records or allowing me to observe practices in your facility.

Section A: Company and product profiles

1. **When was your company established?**

……

2. **What sort of products do you produce?**

……..

3. **When did you start making kombucha?**

……...

4. **How many brands of Kombucha do you make?**

……..

5. **How much Kombucha do you make?**

Sub-question	**Time frame**	**Amount (Litres)**
A	**Daily**	
B	**Weekly**	
C	**Monthly**	

6. **In what volumes do you pack and sell?**

Sub-question	**Volume**	**Tick correct response (√)**
A	**300 ml**	
B	**500 ml**	
C	**1 L**	
D	**Other: specify …….**	

7. **How much do you sell each volume of Kombucha?**

Sub-question	Volume	Cost (UGX) per unit
A	**300 ml**	
B	**500 ml**	
C	**1 L**	
D	**Other: specify …….**	

8. **Where do you distribute your products?**

……

9. Do you have any certified products (Kombucha or other products) on the market?

(a) Yes

(b) No

10. If you said yes to 9, list the brands of your products (Kombucha and other products) that are certified

Product Type	Brand name

11. Can I see a sample of your certified product (*Kombucha only*) and any proof of certification? (Please ask for a sample to see and records or letters that show)

(a) Products not seen but processor claims they are certified: …….

(b) Sample seen with UNBS mark: …….

(c) UNBS letter of certification seen: …….

(d) Any other relevant comment/observation ……

……

12. If you said no to 9, list the brands of your products (Kombucha and other products) that are not certified

Product Type	Brand name

13. If you said no to 9, why are your products not certified? ……

……

Section B: Food safety knowledge

Ticking against the most applicable answer/response and note down any key own remarks given by the respondent or upon probing by the interviewer

Q.No.	Questions	Response/ answer		Additional remarks
14	Is it necessary to have your product certified by UNBS?	YES	NO	
15	Hand washing prior to processing contributes to Kombucha safety	YES	NO	
16	Hand washing after touching money is important for Kombucha safety	YES	NO	
17	Hand washing after using washrooms is important for Kombucha safety	YES	NO	
18	Hand washing after touching the body is	YES	NO	

	important for Kombucha safety			
19	Hand washing after using the phone is important for Kombucha safety	YES	NO	
20	Hand washing hands after each break is important for Kombucha safety	YES	NO	
21	Hand washing after handling garbage is important for Kombucha safety	YES	NO	
22	Sanitizing utensils increases the risk of Kombucha contamination	YES	NO	
23	Washing utensils with detergent makes them sterile	YES	NO	
24	Eating and drinking during processing increases the risk of Kombucha contamination	YES	NO	
25	Diarrhea, vomiting and stomach pain arise from drinking Kombucha made unhygienically	YES	NO	
26	Microorganisms are found on the skin, hair and hands of processors and they are potential disease causers if they get into Kombucha	YES	NO	

27	The use of clean and well stored raw materials is vital for Kombucha safety	YES	NO	
28	Pathogens change the sensory properties of Kombucha	YES	NO	
27	Monitoring of water quality is important in ensuring Kombucha safety	YES	NO	
28	What is GMP/GHP program?	KNOWS	NOT SURE/DOESN'T KNOW	
29	What is a HACCP plan?	KNOWS	NOT SURE/DOESN'T KNOW	
30	What do you understand by a product standard/ specification?	KNOWS	NOT SURE/DOESN'T KNOW	
31	Does Uganda have a product specification for Kombucha?	YES	NO	
32	What is the standard/spec for Kombucha?	KNOWS	NOT SURE/DOESN'T KNOW	

Section C: Food Processing Practices

Ticking against the most applicable answer/response. (Ensure to confirm practice by personally checking or observing processes or request for any signed records to verify)

S/N	Questions	Response		Practice Confirmed visually by researcher	How was practice confirmed	Researcher was unable to, or did not, confirm practice
33	Do you have a foot bath at the entry to the facility?	YES	NO			
34	Do you check the length and cleanliness of the nails of the processors?	YES	NO			
35	Do you ensure workers wear proper head gear during processing	YES	NO			
36	Do you ensure workers wear closed shoes during processing?	YES	NO			
37	Do processors remove jewelry and other accessories before processing?	YES	NO			
38	Do you ensure workers wear separate clothes specific for processing?	YES	NO			

39	Are processors are examined for contagious diseases	YES	NO			
40	Workers wash and sanitize their hands before and during work	YES	NO			
41	Do you sanitize of utensils before processing	YES	NO			
42	Do you sanitize of utensils after processing	YES	NO			
43	Do you sanitize packaging material before use	YES	NO			
44	Is the facility vermin proof storage facility?	YES	NO			
45	Do you use treated water for Kombucha processing?	YES	NO			
46	Do you use objective methods (e.g. pH, TA, bubbling ceases) to determine if Kombucha is ready? Please give examples	YES	NO			
47	Do you adequately clean packaging materials (use soap, clean water and sanitizer)	YES	NO			

48	Do you use running water/regularly change water for washing used bottles and cups	YES	NO			
49	Do you wash utensils after Kombucha processing	YES	NO			
50	Do you store utensils in a clean area separate from raw materials	YES	NO			
51	Do you use Kombucha preparation utensils for other purposes?	YES	NO			
52	Do you refrigerate Kombucha during storage, before sale, transportation and marketing of the product	YES	NO			
53	Do you dispose garbage in a covered garbage receptacle	YES	NO			
54	Do you have /follow a Kombucha specification, if YES, state it	YES	NO			
55	Do you follow a Hazard Analytical Critical Control Point (HACCP) plan?	If 'YES' has the HACCP plan been verified? Provide the records for	NO			

		verification of the practices				

Section D: Kombucha product processing

Please use the answer sheet provided for this section

56 Could you briefly describe the process you follow in making Kombucha (Including; raw materials) processing ingredients (additives or preservatives used and amounts per unit volume), processing conditions (time and temperature) followed, Quality control tests done during processing and when (e.g pH, TTS among others).

57 Do you carry out any quality or safety tests on your products? If yes, how often? What sort of tests do you carry out? Can I have a look at the records (verification)

Type of test	How often it is done	Target results	Records verification

Section E: Sample collection:

I would like to take a sample of your product with me for analysis.

Sample code: …… **Product:……**

Brand: ……. **Batch/Lot number: ……**

Date: ……

Thanks for your involvement

Appendix 5: A summarized write-up of the HACCP key Steps and principles that will guide in the development of Kombucha HACCP plan (UNBS: 2017)

Developing a HACCP Plan

HACCP plans are specific to the product categories and processes that have to be developed.

In the development of a HACCP plan, five preliminary steps need to be accomplished before the application of the HACCP principles to a specific products and process. The five preliminary steps include;

a) Assembly of the HACCP Team

The first step in developing a HACCP plan is to assemble a HACCP team consisting of individuals who have specific knowledge and expertise appropriate to the product and process. It is the team's responsibility to develop the HACCP plan. The team should be multi-disciplinary and include individuals from areas such as engineering, production, sanitation, quality assurance, and food microbiology, food science and technology and food safety. The team should also include local personnel who are involved in the operation as they are more familiar with the variability and limitations of the operation. The HACCP team may need assistance from outside experts such as academia, regulatory bodies such and conformity assessment bodies who are knowledgeable in the potential biological, chemical and/or physical hazards associated with the product and the process.

Due to the technical nature of the information required for hazard analysis, it is recommended that experts who are knowledgeable in the food process should either participate in or verify the completeness of the hazard analysis and the HACCP plan. Such individuals should have the knowledge and experience to correctly: (a) conduct a hazard analysis; (b) identify potential hazards; (c) identify hazards which must be controlled; (d) recommend controls, critical limits, and procedures for monitoring and verification; (e) recommend appropriate corrective actions when a deviation occurs; (f) recommend research related to the HACCP plan if important information is not known; and (g) validate the HACCP plan.

b) Description of the food and its distribution

The HACCP team first describes the food (Kombucha). This consists of a general description of the food, ingredients, and processing methods. The method of distribution should be described

along with information on whether the food is to be distributed frozen, refrigerated, or at ambient temperature.

c) Description of the intended use and consumers of the food

Describe the normal expected use of the food. The intended consumers may be the general public or a particular segment of the population (e.g., infants, immunocompromised individuals, elderly, among others).

d) Development of specific flow diagram which describes the process of the product

The purpose of a flow diagram is to provide a clear, simple outline of the steps involved in the process. The scope of the flow diagram must cover all the steps in the process which are directly under the control of the establishment. In addition, the flow diagram can include steps in the food chain which are before and after the processing that occurs in the establishment. Also, a simple schematic of the facility is often useful in understanding and evaluating product and process flow.

e) Onsite conformation of the flow diagram

The HACCP team should perform an on-site review of the operation to verify the accuracy and completeness of the flow diagram. Modifications should be made to the flow diagram as necessary and documented in following the physical processes in the food industry.

After these five preliminary tasks have been completed, the seven principles of HACCP are applied and these include;

1. Conducting of a hazard analysis.

After addressing the preliminary steps discussed above, the HACCP team conducts a hazard analysis and identifies all hazards including biological, chemical or physical hazards and appropriate control measures.

A thorough hazard analysis is the key to preparing an effective HACCP plan. If the hazard analysis is not done correctly and the hazards warranting control within the HACCP system are not identified, the plan will not be effective regardless of how well it is followed.

The hazard analysis and identification of associated control measures accomplish three objectives: Those hazards and associated control measures are identified. The analysis may identify needed

modifications to a process or product so that product safety is further assured or improved. The analysis provides a basis for determining Critical Control Points in Principle 2.

The process of conducting a hazard analysis involves two stages. The first, hazard identification, can be regarded as a brain storming session. During this stage, the HACCP team reviews the ingredients used in the product, the activities conducted at each step in the process and the equipment used, the final product and its method of storage and distribution, and the intended use and consumers of the product. Based on this review, the team develops a list of potential biological, chemical or physical hazards which may be introduced, increased, or controlled at each step in the production process.

After the list of potential hazards is assembled, stage two, the hazard evaluation, is conducted. In stage two of the hazard analysis, the HACCP team decides which potential hazards must be addressed in the HACCP plan. During this stage, each potential hazard is evaluated based on the severity of the potential hazard and its likely occurrence. Severity is the seriousness of the consequences of exposure to the hazard. Considerations of severity (for example, impact of sequelae, and magnitude and duration of illness or injury) can be helpful in understanding the public health impact of the hazard. Consideration of the likely occurrence is usually based upon a combination of experience, epidemiological data, and information in the technical literature. When conducting the hazard evaluation, it is helpful to consider the likelihood of exposure and severity of the potential consequences if the hazard is not properly controlled. In addition, consideration should be given to the effects of short term as well as long-term exposure to the potential hazard. Such considerations do not include common dietary choices which lie outside of HACCP. During the evaluation of each potential hazard, the food, its method of preparation, transportation, storage and persons likely to consume the product should be considered to determine how each of these factors may influence the likely occurrence and severity of the hazard being controlled. The team must consider the influence of likely procedures for food preparation and storage and whether the intended consumers are susceptible to a potential hazard. However, there may be differences of opinion, even among experts, as to the likely occurrence and severity of a hazard. The HACCP team may have to rely upon the opinion of experts who assist in the development of the HACCP plan.

Hazards identified in one operation or facility may not be significant in another operation producing the same or a similar product. For example, due to differences in equipment and/or an effective maintenance program, the probability of metal contamination may be significant in one facility but not in another. A summary of the HACCP team deliberations and the rationale developed during the hazard analysis should be kept for future reference. This information will be useful during future reviews and updates of the hazard analysis and the HACCP plan.

Upon completion of the hazard analysis, the hazards associated with each step in the production of the food should be listed along with any measure(s) that are used to control the hazard(s). On the other hand, more than one hazard may be addressed by a specific control measure.

2. Determination of critical control points (CCPs) and OPRPs.

A critical control point is defined as a step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level. The potential hazards that are reasonably likely to cause illness or injury in the absence of their control must be addressed in determining CCPs. Operational Pre-requisite program (OPRP) is a control measure for specific hazards that do not have specific critical limits.

Complete and accurate identification of CCPs is fundamental to controlling food safety hazards. The information developed during the hazard analysis is essential for the HACCP team in identifying which steps in the process are CCPs. One strategy to facilitate the identification of each CCP is the use of a CCP decision tree.

Examples of CCPs may include: thermal processing, chilling, testing ingredients for chemical residues, product formulation control, and testing product for metal contaminants. CCPs, OPRPs and PRPs must be carefully developed and documented.

3. Establishment of critical limits.

A critical limit is a maximum and/or minimum value to which a biological, chemical or physical parameter must be controlled at a CCP to prevent, eliminate or reduce to an acceptable level the occurrence of a food safety hazard. A critical limit is used to distinguish between safe and unsafe operating conditions at a CCP.

Each CCP will have one or more control measures to assure that the identified hazards are prevented, eliminated or reduced to acceptable levels. Each control measure has one or more associated critical limits. Critical limits may be based upon factors such as: temperature, time, physical dimensions, humidity, moisture level, water activity (aw), pH, titratable acidity, salt concentration, available chlorine, viscosity, preservatives, or sensory information such as aroma and visual appearance. Critical limits must be scientifically based. For each CCP, there is at least one criterion for food safety that is to be met. An example of a criterion is a specific lethality of a cooking process such as a 5D reduction in Salmonella. The critical limits and criteria for food safety may be derived from sources such as regulatory standards and guidelines, literature surveys, experimental results, and experts.

4. Establishment of monitoring procedures.

Monitoring is a planned sequence of observations or measurements to assess whether a CCP is under control and to produce an accurate record for future use in verification. Monitoring serves three main purposes. First, monitoring is essential to food safety management in that it facilitates tracking of the operation. If monitoring indicates that there is a trend towards loss of control, then action can be taken to bring the process back into control before a deviation from a critical limit occurs. Second, monitoring is used to determine when there is loss of control and a deviation occurs at a CCP, i.e., exceeding or not meeting a critical limit. When a deviation occurs, an appropriate corrective action must be taken. Third, it provides written documentation for use in verification.

An unsafe food may result if a process is not properly controlled and a deviation occurs. Because of the potentially serious consequences of a critical limit deviation, monitoring procedures must be effective. Ideally, monitoring should be continuous, which is possible with many types of physical and chemical methods. For example, the temperature and time for the scheduled thermal process of low-acid canned foods is recorded continuously on temperature recording charts. If the temperature falls below the scheduled temperature or the time is insufficient, as recorded on the chart, the product from the retort is retained and the disposition determined as in Principle 5. Likewise, pH measurement may be performed continually in fluids or by testing each batch before processing. There are many ways to monitor critical limits on a continuous or batch basis and

record the data on charts. Continuous monitoring is always preferred when feasible. Monitoring equipment that must be carefully calibrated for accuracy.

Assignment of the responsibility for monitoring is an important consideration for each CCP. Specific assignments will depend on the number of CCPs and control measures and the complexity of monitoring. Personnel who monitor CCPs are often associated with production (e.g., line supervisors, selected line workers and maintenance personnel) and, as required, quality control personnel. Those individuals must be trained in the monitoring technique for which they are responsible, fully understand the purpose and importance of monitoring, be unbiased in monitoring and reporting, and accurately report the results of monitoring. In addition, employees should be trained in procedures to follow when there is a trend towards loss of control so that adjustments can be made in a timely manner to assure that the process remains under control. The person responsible for monitoring must also immediately report a process or product that does not meet critical limits.

All records and documents associated with CCP monitoring should be dated and signed or initialed by the person doing the monitoring.

When it is not possible to monitor a CCP on a continuous basis, it is necessary to establish a monitoring frequency and procedure that will be reliable enough to indicate that the CCP is under control. Statistically designed data collection or sampling systems lend themselves to this purpose.

Most monitoring procedures need to be rapid because they relate to on-line, "real-time" processes and there will not be time for lengthy analytical testing. Examples of monitoring activities include: visual observations and measurement of temperature, time, pH, and moisture level.

Microbiological tests are seldom effective for monitoring due to their time-consuming nature and problems with assuring detection of contaminants. Physical and chemical measurements are often preferred because they are rapid and usually more effective for assuring control of microbiological hazards. For example, the safety of Kombucha is based upon measurements of time and temperature of heating of water and the ingredients like sugar and tea rather than testing the heated Kombucha to assure the absence of surviving pathogens.

With certain foods, processes, ingredients, or imports, there may be no alternative to microbiological testing. However, it is important to recognize that a sampling protocol that is

adequate to reliably detect low levels of pathogens is seldom possible because of the large number of samples needed. This sampling limitation could result in a false sense of security by those who use an inadequate sampling protocol. In addition, there are technical limitations in many laboratory procedures for detecting and quantitating pathogens and/or their toxins.

5. Establishment corrective actions.

Where there is a deviation from established critical limits, corrective actions are necessary. Therefore, corrective actions should include the following elements: (a) determine and correct the cause of non-compliance; (b) determine the disposition of non-compliant product and (c) record the corrective actions that have been taken. Specific corrective actions should be developed in advance for each CCP and included in the HACCP plan. As a minimum, the HACCP plan should specify what is done when a deviation occurs, who is responsible for implementing the corrective actions, and that a record will be developed and maintained of the actions taken. Individuals who have a thorough understanding of the process, product and HACCP plan should be assigned the responsibility for oversight of corrective actions. As appropriate, experts may be consulted to review the information available and to assist in determining disposition of non-compliant product.

6. Establishment of verification procedures.

Verification is defined as those activities, other than monitoring, that determine the validity of the HACCP plan and that the system is operating according to the plan.

One aspect of verification is evaluating whether the facility's HACCP system is functioning according to the HACCP plan. An effective HACCP system requires little end-product testing, since sufficient validated safeguards are built in early in the process.

Another important aspect of verification is the initial validation of the HACCP plan to determine that the plan is scientifically and technically sound, that all hazards have been identified and that if the HACCP plan is properly implemented these hazards will be effectively controlled. Information needed to validate the HACCP plan often include (1) expert advice and scientific studies and (2) in-plant observations, measurements, and evaluations. For example, validation of the heating/boiling of water an ingredient in Kombucha processing should include the scientific justification of the heating times and temperatures needed to obtain an appropriate destruction of

pathogenic microorganisms and studies to confirm that the conditions of heating will deliver the required time and temperature.

Subsequent validations are performed and documented by a HACCP team or an independent expert as needed. For example, validations are conducted when there is an unexplained system failure; a significant product, process or packaging change occurs; or new hazards are recognized.

In addition, a periodic comprehensive verification of the HACCP system should be conducted by an unbiased, independent authority. Such authorities can be internal or external to the food operation. This should include a technical evaluation of the hazard analysis and each element of the HACCP plan as well as on-site review of all flow diagrams and appropriate records from operation of the plan. A comprehensive verification is independent of other verification procedures and must be performed to ensure that the HACCP plan is resulting in the control of the hazards. If the results of the comprehensive verification identify deficiencies, the HACCP team modifies the HACCP plan as necessary.

Verification activities are carried out by individuals within a company, third party experts, and regulatory agencies. It is important that individuals doing verification have appropriate technical expertise to perform this function. Such activities include sampling and testing plan for the different products and analysis of the obtained results to be used in improving the HACCP plan.

7. Establish record-keeping and documentation procedures.

Generally, the HACCP team leader should ensure the following are maintained in an up to date versions:

a. A summary of the hazard analysis, including the rationale for determining hazards and control measures.

b. The HACCP Plan

Listing of the HACCP team and assigned responsibilities, Description of the food, its distribution, intended use, and consumer, verified flow diagram, HACCP Plan Summary Table that includes information for, Steps in the process that are CCPs, The hazard(s) of concern, Critical limits, Monitoring records, Corrective actions records, Verification procedures and schedules, Record-keeping procedures

c. Support documentation such as validation records.

d. Records that are generated during the operation of the plan.

Appendix 6: Analyzed data on Kombucha product safety and quality performance

Table 10: Microbial counts of Kombucha products and their conformance with specification

	Microbial counts (CFU/ml)			
Product	Staph (CFU/ml)	E. coli (MPN/ml)	Salmonella spp (25ml)	YM (CFU/ml)
C1	<1	<1	ND	<1
C2	<1	0	ND	<1
C3	<1	0	ND	TNTC
C4	<1	<1	ND	TNTC
UC1	<1	0	ND	<1
UC2	<1	0	ND	<1
UC3	<1	0	ND	TNTC
UC4	<1	<1	ND	TNTC
Conformance with Specification				
Specification	Absent	Absent	Absent in 25 ml	100 Max.
% Conformance	100	100	100	50
% Non-conformance	0	0	0	50
Reference Specification			US 2037: 2019	

N = 8 (1 samples per product). Values for means ± standard deviations not applicable. Staph = Staphylococcus aureus, YM= Yeasts and Molds, TNTC= Too numerous to count, ND= Not detected, U=Certified product, UC=Uncertified products, Max. = Maximum.

Table 11: Physicochemical properties of Kombucha products and their conformance with specifications

	Physicochemical attributes	
Product	Alcohol content (%v/v)	Acidity as Acetic acid (acetic acid, g/L)
C1	0.07	0.3
C2	0.07	1
C3	5	0.2
C4	5.3	1
UC1	2	0.5
UC2	2.3	0.2
UC3	4.2	2
UC4	0.07	0.2
Conformance with Specification		

Specification	(0.5 Maxi. for Non-alcoholic) and (0.6 - 15 - Alcoholic)	Acidity as Acetic acid (acetic acid, g/L)
% Conformance	62.5	100
% Non- conformance	37.5	0
Reference Specification	US 2037: 2019	

N= 8 (1 samples per product). Values are means ± standard deviations, C = certified product, UC = Uncertified product

Table 12: Heavy metal limits of Kombucha products and their conformance with specifications

	Heavy metal limits			
Product	**Lead (mg/L)**	**Arsenic (mg/l)**	**Mercury (mg/l)**	**Cadmium (mg/l)**
C1	<0.05	<0.05	<0.1	<0.002
C2	<0.05	<0.05	<0.1	<0.002
C3	<0.05	<0.05	<0.1	<0.002
C4	<0.05	<0.05	<0.1	<0.002
UC1	<0.05	<0.05	<0.1	<0.002
UC2	<0.05	<0.05	<0.1	<0.002
UC3	<0.05	<0.05	<0.1	<0.002
UC4	<0.05	<0.05	<0.1	<0.002
Conformance with Specification				
Specification	0.05 Maximum	0.05 Maximum	0.001 Maximum	0.003 Maximum
% Conformance	100	100	100	100
% Non-conformance	0	0	0	0
Reference Specification	US 2037: 2019			

N= 8 (1 samples per product). Values are means ± standard deviations, C = certified product, UC = Uncertified product

Appendix 7: Relationships between product safety certification and knowledge practices levels of the Kombucha processors

Table 13: Relationship between Certification and Knowledge and practices on level

		Knowledge level		Practices		
		Fairly Good	Fairly Poor	Fairly Good	Very Good	Very Good
Certification	Uncertified	1	3	1	1	2
	Certified	1	3	0	0	4
		Chi sq =0.000; p=0.786			**Chi sq** =2.667; p=0.269	

Table 14: Relationship between Kombucha certification and product conformance with specification

		Yeast and molds		Alcohol content		Overall conformance	
		Fail	Pass	Fail	Pass	Fail	Pass
Certification	Uncertified	2	2	2	2	3	1
	Certified	2	1	1	3	3	1
Total		Chi sq =0.000; p=0.757		Chi sq =0.533; p=0.465		Chi sq =0.000; p=0.786	

Table 15: Relationship between food safety knowledge and product conformance with specification

		Yeast and molds		Alcohol content		Overall conformance	
		Fail	Pass	Fail	Pass	Fail	Pass
Knowledge level	Fairly Good	2	1	1	1	2	0
	Very Good	2	2	2	4	4	2
Total		Chi sq =2.667; p=0.106		Chi sq =0.178; p=0.673		Chi sq =0.889; p=0.346	

Table 16: Relationship between food safety practices and product conformance with specification

		Yeast and molds		Alcohol content		Overall conformance	
		Fail	Pass	Fail	Pass	Fail	Pass
Practices	Fairly Poor	1	0	1	0	1	0
	Fairly Good	0	1	0	1	0	1
	Very Good	3	3	3	3	5	1
Total		Chi sq=2.000; p=0.368		Chi sq =2.311; p=0.315		Chi sq =3.556; 0.169	

Appendix 8: Raw data tables from the Field

Raw data on Kombucha company profile

Company name	Location	Address	1.Company year of establishment	2.What sort of products do you produce?	3.When did you start making kombucha?	Years producing Kom	Number of Kombuchs Brands	Quantity produced Daily (L)	ADaily	BWeekly	CMonthly	A300ml	Price of 300ml	Areas of distribution	9. certified Kombuchproducts on the market?	AYes	10. If yes, list the brands of your products	Product Type	(a)Products not seen but processor claims they are certified	(b)Sample seen with UNBS mark	(c)UNBS letter of certification seen
C1	KAWEMPE BOMBO ROAD	PLOT 32 - 33 BOMBO ROAD	2005	FOODS AND BEVERAGES	12/07/1905	1	1					YES		COUNTRYWIDE	YES	YES		KOMBUCHA			YES
C2	NTUNGAMO ITOJO	ITOJO KASHSHA	2008	NON ALCOHOLIC KOMBUCHA	2018	3	1	5000	5000L			YES	1000	COUNTRYWIDE	YES	YES		NON ALCOHOLIC			YES
C3	NABWERU KAWEMPE	PLOT 923 MUTUBA 2	2017	JAJJA & LEO	2018	3	1					YES	800	COUNTYWIDE	YES	YES		KOMBUCHA, BEER, WINE		YES	YES
C4	NANSANA	NANSANA	2019	KOMBUCHA	2019	2	2	15000	150	100000	3000	YES	750	ALBERTINE	YES	YES		ALCOHOLIC			

	EAST 2 B								00L		000			REGION							
UC 1	MITYANA DISTRICT	KANNAMBBA-MITYANA TOWN	2020	KOMBUCHA DRINK	2021	0.5	1	4000	40000L			YES	1000	KAMPALA	NO	NO			YES		
UC 2	KIBAALE	KIBAALE	2018	FOREST PLANT	2018	3	1	100				YES	700	KIBAALE		NO					
UC 3	KASESE	KASESE	2017	REHEEMA	2017	4	1	200				YES	800	KASESE		NO					
UC 4	KAKOBAMBARARA	KAKOBA CENTRAL CELL	2018	ENTARESANA	2018	3	2	5000	50000L			YES	1000					NON ALCOHOLIC			YES

Raw data on knowledge levels of Kombucha processor

Company name	14 Is it necessary to have your product certified by UNBS?	15 Hand washing prior to processing contributes to Kombucha safety	16 Hand washing after touching money is important for Kombucha safety	17 Hand washing after using washrooms is important for Kombucha safety	18 Hand washing after touching the body is important for Kombucha safety	19 Hand washing after using the phone is important for Kombucha safety	20 Hand washing hands after each break is important for Kombucha safety	21 Hand washing after handling garbage is important for Kombucha safety	22 Sanitizing utensils increases the risk of Kombucha contamination	23 Washing utensils with detergent makes them sterile	24Eating and drinking during processing increases the risk of Kombucha contamination	25 Diarrhea, vomiting and stomach pain arise from drinking Kombucha made unhygienically	26 Micro organisms are found on the skin, hair and hands of processors and they are potential disease causers if they get into Kombucha	27 The use of clean and well stored raw materials is vital for Kombucha safety	28 Pathogens change the sensory properties of Kombucha	27 Monitoring of water quality is important in ensuring Kombucha safety	28 What is GMP/GHP program?	29 What is a HACCP plan?	30 What do you understand by a product standard/ specification?	31 Does Uganda have a product specification for kombucha?	32 What is the standard/spec for kombucha?
C1	YES	YES	YES	YES	YES	YES	YES	YES	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
C2	YES	YES	YES	YES	NO	NO	NO	YES	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
C3	YES	YES	YES	YES	YES	NO	YES	YES	YES	YES	YES	NO	NO	YES	YES	YES	NO	NO	YES	YES	YES

C4	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
UC1	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	YES	YES	YES	YES				NO	YES	YES	NO
UC2	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	NO
UC3	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NO	NO	YES	YES	YES	NO	NO	NO	YES	NO
UC4	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	YES	YES	YES	YES	YES	YES	NO	NO	NO	YES	NO

Raw data on practice levels for Kombucha processor

Company name	33 Do you have a foot bath at the entry to the facility?	34 Do you check the length and cleanliness of the nails of the processors?	35 Do you ensure workers wear proper head gear during processing	36 Do you ensure workers wear closed shoes during processing?	37 Do processors remove jewelry and other accessories before processing?	38 Do you ensure workers wear separate clothes specific for processing?	39 Are processors are examined for contagious diseases	40 Workers wash and sanitize their hands before and during work	41 Do you sanitize of utensils before processing	42 Do you sanitize of utensils after processing	43 Do you sanitize packaging material before use	44 Is the facility verminproof storage facility?	45 Do you use treated water for Kombucha processing?	46 Do you use objective methods (e.g. pH, TA, bubbling ceases) to determine if Kombucha is ready? Please give examples	47 Do you adequately clean packaging materials (use soap, clean water and sanitizer)	48 Do you use running water/ regularly change water for washing used bottles and cups	49 Do you wash utensils after Kombucha processing	50 Do you store utensils in a clean area separate from raw materials	51 Do you use Kombucha preparation utensils for other purposes?	52 Do you refrigerate Kombucha during storage, before sale, transportation and marketing of the product	53 Do you dispose garbage in a covered garbage receptacle	54 Do you have /follow a Kombucha specification, if YES, state it	55 Do you follow a Hazard Analytical Critical Control Point (HACCP) plan?
C1	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	YES	YES	YES	YES

C2	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	YES	YES	YES	YES	YES	YES
C3	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	YES	NO	YES	YES	YES	YES	NO	NO	YES	YES	YES
C4	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	YES	
UC1	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	YES	NO	NO	NO	YES	YES	YES	NO	NO	NO	NO
UC2	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	YES	YES	NO	NO	NO	NO	NO	NO
UC3	NO	YES	YES	YES	NO	YES	NO	YES	NO	YES	NO	YES	YES	NO	YES	NO	YES	NO	NO	YES	NO	NO	NO
UC4	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	YES	NO	NO	YES	NO	NO

Raw data tables for alcoholic Kombucha samples tested pre and post HACCP plan implementation

Pre HACCP plan data

	Test parameter and results									
Sample code	**Alcohol content (%v/v) - 0.5 (Maximum) for Non-alcoholic Kombucha, 0.6 - 15 (Range) Alcoholic Kombucha**	**Acidity as Acetic acid (acetic acid, g/L) - 2 (Maximum)**	**Lead (mg/L)-0.05 (Maximum)**	**Cadmium (mg/l)-0.003 (Maximum)**	**Arsenic (mg/l)-0.05 (Maximum)**	**Mercury (mg/l) 0.001 (Maximum)**	**Escherichia coli (CFU/ml)**	**Yeast and moulds (CFU/ml)**	***Staphylococcus aureus* (CFU/ml)**	**Salmonella (/25ml)**
ai	2	0.5	<0.05	<0.002	<0.05	<0.001	<1	TNTC	<1	Not detected
aii	2.1	0.5	<0.05	<0.002	<0.05	<0.001	<1	TNTC	<1	Not detected
bi	2	0.5	<0.05	<0.002	<0.05	<0.001	<1	TNTC	<1	Not detected
bii	2	0.5	<0.05	<0.002	<0.05	<0.001	<1	TNTC	<1	Not detected

(ai……bii are sample codes), (n= 2 in replicates)

Post HACCP plan data

	Test parameter and results									
Sample code	**Alcohol content (%v/v) - 0.5 (Maximum) for Non-alcoholic Kombucha, 0.6 - 15 (Range) Alcoholic Kombucha**	**Acidity as Acetic acid (acetic acid, g/L) - 2 (Maximum)**	**Lead (mg/L)- 0.05 (Maximum)**	**Cadmium (mg/l)- 0.003 (Maximum)**	**Arsenic (mg/l)- 0.05 (Maximum)**	**Mercury (mg/l) 0.001 (Maximum)**	**Escherichia coli (CFU/ml)**	**Yeast and moulds (CFU/ml)**	***Staphylococcus aureus* (CFU/ml)**	**Salmonella (/25ml)**
Ai	1.1	0.9	<0.05	<0.002	<0.05	<0.001	<1	<1	<1	Not detected
Aii	1.1	0.9	<0.05	<0.002	<0.05	<0.001	<1	<1	<1	Not detected
Bi	1.1	0.9	<0.05	<0.002	<0.05	<0.001	<1	<1	<1	Not detected
Bii	1.0	0.9	<0.05	<0.002	<0.05	<0.001	<1	<1	<1	Not detected
Ci	1.1	0.9	<0.05	<0.002	<0.05	<0.001	<1	<1	<1	Not detected
Cii	1.1	0.9	<0.05	<0.002	<0.05	<0.001	<1	<1	<1	Not detected
Di	1.0	0.9	<0.05	<0.002	<0.05	<0.001	<1	<1	<1	Not detected
Dii	1.1	0.9	<0.05	<0.002	<0.05	<0.001	<1	<1	<1	Not detected

(Ai……Dii are sample codes), (n= 4 in replicates)

Relevant data output on means of samples picked during HACCP implementation and validation

Paired sample Test

	Paired Differences							
	Mean	Std. Deviation	Std. Error Mean	95% confidence interval of the Difference		t	df	Sig. (2-tailed)
				Lower	Upper			
Pair 1 Alcohol content before HACCP – Alcohol content after	.40000	.08165	.04082	.27008	.52992	9.798	3	.002

Paired sample Test

	Paired Differences							
	Mean	Std. Deviation	Std. Error Mean	95% confidence interval of the Difference		t	df	Sig. (2-tailed)
				Lower	Upper			
Pair 1 Acidity as acetic acid before HACCP – Acidity as acetic acid after	.40000	.08165	.04082	.27008	.52992	9.798	3	.002

Appendix 9: Photos taken during the field data collection

Photo 1: A water treatment system used for treating process water in a Kombucha processing plant in Wakiso, Uganda

Photo 2: Kombucha packaging bottles in store

Photo 3: Designated dispatch area at one of the Kombucha processing facilities in Kampala, Uganda

Appendix 10: Photos taken in the field during HACCP system training

Photo 4: Participants during the HACCP training at Hotel B plus, Mbarara

Photo 5: HACCP system training in Mbarara

Photo 6: Collaborative participation in development of a HACCP plan for Kombucha

Appendix 11: Photos taken in the field during HACCP plan verification and validation

Photo 7: A foot bath (left) at the processing area entry and a Kombucha boiling (Right) site at the industry respectively

Photo 8: Inspection of the industry for hygiene prior to Kombucha production

Photo 9: A and B show Kombucha cooling in open drums and fermenting in cover drums at the processing plant

Photo 10: Kombucha in holding tanks ready for packaging

Photo 11: Kombucha packaging site in the processing plan

Photo 12: Packaged Kombucha in the processing plant store ready to the market