

Kombucha Production in Uganda: Quality Aspects and Compliance with Standards

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ABSTRACT

Kombucha is a mildly sweet and acidic fermented tea beverage. Its production and consumption in Uganda have expanded dramatically as a result of its purported nutritional and health benefits. However, there has been little research into the quality and safety of commercially produced Kombucha in Uganda. This study evaluated the quality and safety of certified (n = 27) and uncertified (n = 16) Kombucha on the market. It also assessed the knowledge and practices of Kombucha processors with certified (n = 4) and uncertified (n = 4) products in Uganda. A HACCP plan for Kombucha processing was also developed and validated with one processor. All products passed the Kombucha requirements for *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., and heavy metals (lead, arsenic, mercury, and cadmium). However, 60.47% of the products did not meet the quality and safety specifications for Kombucha failing to meet the acidity (n = 3), alcohol content (n = 14), and yeasts and molds (n = 15). The majority of the processors (n = 6) had very good scores (> 75%) for knowledge and practices related to food safety but did not know the importance of sanitizing equipment. Half of the processors did not know about HACCP, its prerequisites, and the Kombucha specification. Four processors did not use objective methods to test product readiness. Half of the processors did not follow the Kombucha specification and had no HACCP plan. A HACCP plan with three CCPs and five CPs was developed and validated. This study, therefore, informs Kombucha processors and regulators on the safety and quality of Kombucha on the market and the importance of HACCP plan development and implementation in achieving product quality.

Keywords: Kombucha, Standards, HACCP, Food Safety, Quality

INTRODUCTION

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; Coelho et al., 2020; Leonarski, et al., 2022). Kombucha production involves the use of a symbiotic

culture of bacteria and yeast (SCOBY) following a fairly standard protocol (Jayabalan et al 2014). The yeast component in the SCOBY comprises *Saccharomyces cerevisiae*, along with other species such as *Candida*, *Saccharomyces*, *Saccharomycoides*, *Schizosaccharomyces*, and *Kluyveromyces* (Jayabalan et al., 2014). The yeasts catalyse the production of ethanol and some flavor compounds such as 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 ethanol to acetic acid and other organic acids, thus increasing the product acidity and limits ethanol content (Greenwalt et al., 2000). *Komagataeibacter xylinus* is also responsible for producing cellulose (from sugars and ethanol) resulting in the formation of a pellicle in which the Acetic Acid Bacteria and yeasts are embedded (Villarreal-Soto et al., 2018).

Fermented beverages are becoming increasingly popular due to their nutritional and health benefits. Kombucha consumption has been linked to a variety of health advantages (Ernst, 2003; Jayabalan et al., 2014). Kombucha health claims include cleansing the blood, lowering blood cholesterol levels, preventing atherosclerosis, lowering blood pressure, and treating inflammatory issues among others (Gharib, 2014; Ernst, 2003). Most of these health benefits have not been proven in human trials, however, some have been proven in animal studies. Kombucha has been shown to have antibacterial, antioxidant, hepatoprotective, and anticancer effects *in vitro* (Gharib, 2014; Ernst, 2003).

Although Kombucha use is growing in Uganda, there is still limited data on sales and consumption. Kombucha is produced on a small, medium, and large scale by several 'known' and 'unknown' enterprises. In this context, 'known' companies are those that are registered and have their products verified by the Uganda National Bureau of Standards (UNBS). The total number of 'known' Kombucha-certified products in Uganda as of 17 August 2020 was twenty-five (25) (UNBS, 2020).

To regulate the manufacture of Kombucha, UNBS developed a specification

(Kombucha drink - Specification US2037: 2019). This standard specifies (i) microorganisms of concern such as yeasts and molds, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp., (ii) heavy metals (Lead, Cadmium, Arsenic, and Mercury), (iii) alcohol content and (iv) acidity as acetic acid (UNBS, 2019). UNBS frequently analyses the quality and safety aspects of Kombucha to ensure that products on the market conform to the standard.

Most of the kombucha on the Ugandan market is produced by small and medium-Enterprises (SMEs). However, most SME's are slow at adopting advanced processing technologies, have underdeveloped food safety control systems, and often do not comply with recommended good manufacturing practices. This is exacerbated by the fact that the majority of local SMEs are run by unqualified employees with limited knowledge of food processing, quality, safety, and hygiene. As a result, most SMEs' products 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*. Before this investigation, there was little information available on the quality and safety of Kombucha in the Ugandan market.

The goals of this study were to (i) determine the quality of Kombucha in the Uganda market, (ii) evaluate Kombucha processors' knowledge and practices, and (iii) develop and test a basic HACCP plan for Kombucha production. The findings will be used to inform processors and regulators about the quality of Kombucha on the market. They will also be used to recommend quality assurance mechanisms that will enable the consumers to enjoy safe and quality Kombucha.

MATERIALS AND METHODS

Study area and design

The study area included central and western districts of Uganda; Kampala, Wakiso, Mityana, Mbarara, Ntungamo, Kibale, and Kasese. These districts were chosen because they house the majority of Kombucha-producing SMEs. A mixed methods research approach with three study designs was used. Firstly, secondary data on the quality and safety aspects of certified and uncertified Kombucha products was obtained from the UNBS database for samples analysed between 2019 and 2020. Secondly, a descriptive cross-sectional design with a survey questionnaire was used to evaluate the knowledge and practices of Kombucha processors. Lastly, a longitudinal and observational study design was used to develop and validate a HACCP plan for Kombucha for one willing Kombucha producer.

Sample size and participants

Secondary data including parameters specified in the Kombucha specification; US 2037: 2019 was obtained from the Uganda National Bureau of Standards (UNBS) database for samples analysed between 2019 and 2020. The database contained results for 200 samples but only 43 samples (27 certified products and 16 uncertified products) had complete data for all parameters and were included in the study. According to UNBS (2020), there were 25 companies with certified products at the time of the study. Since the number of companies with uncertified products was not known it was assumed to be equal to that of those with certified products (thus giving a total of $n = 50$). Using an online sample calculator (Raosoft, 2004), a margin of error of 5%, a confidence level of 95%, an estimated population size of 50, and a non-response rate of 10%, the sample size was estimated as 50 (with 25 having certified products). It was

planned to interview 50 processors (1 processor per company \times 50 companies = 50) to ascertain their knowledge and practices concerning 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. However, a number of companies declined to participate in the study while some were out of production at the time of the study due to challenges associated with the COVID-19 pandemic. In the end, only eight (8) companies agreed to participate in the study.

Analysis of quality and safety parameters of Kombucha

Data on the quality of Kombucha was obtained from the UNBS. The data set comprised 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* spp, *Salmonella* spp), and heavy metals namely; lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (Ar). The alcohol content was determined by the specific gravimetric method (UNBS, 2014), and acidity was determined by UNBS (1998). *E. coli*, yeasts and molds, and *Staphylococcus* spp counts were determined following UNBS (2012), UNBS (2008), and UNBS (2014) standard methods, respectively. Detection of *Salmonella* spp. was done according to UNBS (2017). 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.



Evaluating knowledge and practices of Kombucha processors

Face-to-face interviews with eight processors (4 from companies with certified products and 4 from companies with uncertified products) were carried out using a researcher-administered questionnaire. The questionnaire used earlier for *Obushera* processors was adopted and modified (Byakika et al., 2019). The questionnaire was composed of sections to capture information on; the company profile, processing of Kombucha, knowledge of basic food safety and hygiene aspects, knowledge of relevant standards/specifications essential for beverage production, and execution of appropriate or recommended practices which were verified by the researcher, HACCP system, Good Hygiene Practices (GHP), Good Manufacturing Practices (GMP) and product certification among others. The questionnaire had provisions for “YES” or “NO” responses concerning knowledge and practice questions.

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

One willing company with uncertified Kombucha was selected to develop and validate a HACCP plan for Kombucha. A detailed recommended HACCP plan was developed following UNBS (2017) and UNBS ISO (2015). The HACCP plan was developed and given to the company for adoption and implementation. The company employees were trained in HACCP system implementation using the Uganda standard for HACCP requirements (US 130:2017). Monitoring of HACCP plan implementation was done through evaluating record keeping and documentation, onsite observations, and product testing for compliance with product standards. HACCP plan validation was done practically by in-plant observation of production processes as stipulated in UNBS

(2017) and UNBS ISO (2015). A baseline to assess product quality and documentation processes was carried out before the 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 described in section 2.3. Two (2) samples per week were picked for analysis for one month.

Statistical analysis

Results of analysis of Kombucha (acidity, alcohol content, microbial counts, and heavy metal analysis) were checked for conformation with the Kombucha specification (pass or fail). Descriptive statistics were 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 the 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). 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.

RESULTS AND DISCUSSION

Quality and safety of Kombucha on the market in Uganda

Table 1 summarizes the conformity assessment of Kombucha from different products and their conformance with specifications. All the samples ($n = 43$) passed the specifications for acetic acid, heavy metals, *Staphylococcus aureus*, *E.coli*, and *Salmonella* spp. However, only 28 and 29 of the samples passed fungal counts and

alcohol content, respectively. Some samples passed yeast and molds but failed the alcohol content and vice versa. This resulted in only 17 of the samples having overall conformance 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, 2019). Therefore, some samples conforming partly to the requirements in the standard did not guarantee total compliance with the specification. Failure on the yeast and molds parameter for both certified and uncertified Kombucha products could be because Kombucha is fermented by a SCOBY containing yeast and most of the products are not pasteurized post-fermentation. Kombucha thus contains leftover yeast from the SCOBY which can continue growing during storage, especially at room temperature thus continuing to catalyse the production of alcohol from fermentable sugars in non-alcoholic Kombucha (Varzakas, 2020), which contributed to the failure of both parameters. Filtration process could be used and considering high temperature short pasteurisation process to eliminate the residual yeast. Continued growth of the SCOBY can also lead to overproduction of acetic acid causing undesirable souring of Kombucha. It is, therefore, important to control microbial growth and this can be done by pasteurizing the final product, 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).

Knowledge and practices of Kombucha processors in Uganda

Characteristics of the Kombucha processors

Table 2 summarizes the major characteristics of the Kombucha processors (n = 8) interviewed. There was an equal proportion of processors with certified and uncertified products. Most of the processors (n = 6) had 2-4 years of 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 the first sample certified by UNBS (cims.unbs.go.ug as of October 2021). Most of the processors (n = 6) had very good knowledge and practices related to food safety. Several studies on the 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 the 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 may know what the standard requires but may opt not to implement the requirements due to a poor attitude, or lack of appreciation of the importance of the specification among other things.

Knowledge of Kombucha processors

Table 3 summarizes the food safety knowledge of the Kombucha processors interviewed. Although the processors had fairly good to very good knowledge scores (Table 2), 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 the certification 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 the 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 while half of the (n = 4) had no knowledge of the prerequisites of HACCP (i.e. GMP/GHP) and a HACCP system as from their responses submitted. In earlier studies by Rossoni and Gaylarde (2000) sodium hypochlorite was reportedly used to sanitize equipment. Its application during cleaning is hence relevant in sanitizing equipment before Kombucha production thus ensuring product safety (Rossoni and Gaylarde, 2000). HACCP system implementation is key in the 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 other food safety systems facilitate trade at national, regional, and international levels as a number of countries adopt similar standard practices in ensuring control of food safety hazards that lead to foodborne illnesses (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 the safety of the final product is not compromised (Pierson, 2012).

Food safety practices of Kombucha processors

Table 4 summarizes the self-reported food safety practices of the Kombucha processors interviewed. All the processors (n = 8) claimed to have good hygiene practices, sanitized utensils, had vermin-proof storage facilities, or used treated water for processing. Food processors are expected to observe proper hygiene and sanitation 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 is an essential control measure for the prevention of pathogens (Ifeadike et al., 2014). 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 a direct quality impact on 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 food from serving points such as rice, beans, and beef (Jeffer et al., 2021; Byakika et al., 2019; Baluka et al., 2015). All of these findings indicate 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 coupled with robust preventive, intervention, and monitoring systems should be strengthened in food production with the SMEs in Uganda.

Hazard Analysis and Critical Control Point plan for Kombucha processing

Technical staff members (n = 4) of one company with uncertified Kombucha were trained in developing and managing a HACCP system. After the training, a process flow diagram (Figure 1) was developed. Each step on the flow diagram was assessed for potential hazards and used to identify critical control points on the HACCP plan. This was important in eliminating significant food safety hazards (UNBS, 2017; UNBS ISO, 2015; Corlett, 1998).

A HACCP plan for the company product was developed (Table 5). Three CCPs and five CPs were identified. The CCPs included: (i) boiling of sugar, tea, and water mixture, (ii) sieving, and (iii) pasteurization of the fermented Kombucha. The CPs included: (i) reception of raw materials and other materials, (ii) storage of raw materials, (iii) fermentation process, (iv) packaging, and (v) storage of finished product. CCPs are important for the complete elimination of significant food safety hazards or for reducing them to acceptable levels that do not compromise consumer safety and health (UNBS, 2017; UNBS ISO, 2015; Corlett, 1998). At these CCPs, critical limits were established as a criterion for separating acceptability from unacceptability for example 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 the loss of control at a CCP would lead to failure in eliminating specified food safety hazards hence affecting the safety of the final product (UNBS ISO, 2015; Corlett, 1998). The HACCP plan was thereafter, given to the company for implementation and validation.

The results of the validation of the developed HACCP plan are shown in Table 6. Before the HACCP plan adoption, the products did not meet the yeasts and molds requirement. This might have been due to the 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 important microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp. and yeast and molds requirements. This was due to the introduction of the pasteurization step as a new CCP during process improvement thus resulting in the removal of yeasts and molds (Byaruhanga and Ndifuna, 2002). After the HACCP plan adoption, the alcohol content was reduced and the acidity increased. The alcohol content after the HACCP plan adoption was reduced due to the 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). Termination of fermentation led to a moderation of the alcohol and acid content, which would ultimately result in improved product shelf stability and 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).

Results for post-HACCP adoption showed improved compliance with the Kombucha specification, implying that the HACCP plan had a significant and positive effect 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 the HACCP plan adoption and overall compliance of the products after the HACCP plan adoption might have been due to acquired knowledge and skills imparted by participants during the HACCP training (Ghafar et al., 2015; Chang et al., 2003). This was evidenced from the improved microbiological quality after HACCP plan implementation. The HACCP plan training and adoption might have improved the industry's food safety system hence leading to products complying with the specification.

CONCLUSION

Despite the good knowledge and practices, only a few 39.53% 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. Although the Uganda 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 provided no post-process treatments are carried out. Therefore, the presence of yeasts in Kombucha may not necessarily amount to a microbial hazard. Their presence is only likely to lead to the production of high amounts of ethanol and early product spoilage. Therefore, this needs

to be taken into consideration when revising the maximum limits for yeasts and molds requirement. A company may have to introduce a pasteurization step post-fermentation or use antifungal preservatives to inactivate the remaining flora from the SCOBY just to ensure product stability and enhanced safety and quality.

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Table 1. Conformity assessment of Kombucha on the Uganda market and its conformance with specifications

Product	Specification requirements (US 2037:2019)										Overall compliance
	<i>Staph</i> (cfu/ml)	<i>E.coli</i> (MPN/ml)	<i>Salmonella</i> Spp. (25ml)	Yeast (cfu/ml)	Alcohol (%v/v)	Acidity (g/L)	Lead (mg/l)	Cadmium (mg/l)	Arsenic (mg/l)	Mercury (mg/l)	
*C1 ⁺	<1	0	ND	<1	<0.07	0.6	0.05	0.002	<0.05	<0.1	P
C2 ⁺	<1	<1	ND	<1	10	0.5	0.05	0.002	<0.05	<0.1	P
*C3	<1	<1	ND	TNTC	2.5	0.1	0.05	0.002	<0.05	<0.1	F
C4 ⁺	<1	0	ND	<1	4.3	0.3	0.05	0.002	<0.05	<0.1	P
C5 ⁺	<1	0	ND	<1	11	0.2	0.05	0.002	<0.05	<0.1	P
C6	<1	0	ND	<1	<0.07	0.2	0.05	0.002	<0.05	<0.1	F
*C7 ⁺	<1	<1	ND	<1	<0.07	0.2	0.05	0.002	<0.05	<0.1	P
*C8 ⁺	<1	<1	ND	21	<0.07	0.4	0.05	0.002	<0.05	<0.1	P
*C9 ⁺	<1	<1	ND	19	<0.07	0.4	0.05	0.002	<0.05	<0.1	P
*C10	<1	0	ND	<1	6	0.8	0.05	0.002	<0.05	<0.1	F
*C11	<1	0	ND	500	0.07	0.4	0.05	0.001	<0.05	<0.1	F
*C12	<1	0	ND	TNTC	0.07	0.4	0.05	0.001	<0.05	<0.1	F
*C13	<1	0	ND	TNTC	0.07	0.4	0.05	0.001	<0.05	<0.1	F
C14	<1	0	ND	TNTC	5.7	0.2	0.05	0.002	<0.05	<0.1	F
*C15 ⁺	<1	0	ND	20	3.7	0.02	0.05	0.002	<0.05	<0.1	P
C16	<1	<1	ND	TNTC	6.9	4	0.05	0.002	<0.05	<0.1	F
C17	<1	<1	ND	TNTC	8.8	5	0.05	0.002	<0.05	<0.1	F
C18	<1	0	ND	<1	0.07	1	0.05	0.002	<0.05	<0.1	F
*C19	<1	0	ND	<1	5.6	1	0.05	0.002	<0.05	<0.1	F
C20	<1	0	ND	TNTC	5	0.2	0.05	0.002	<0.05	<0.1	F
C21 ⁺	<1	<1	ND	<1	<0.07	0.4	0.05	0.002	<0.05	<0.1	P
*C22	<1	<1	ND	<1	1.8	16	0.5	0.05	<0.002	<0.05	F
C23 ⁺	<1	<1	ND	35	5.9	0.9	0.05	0.002	<0.05	<0.1	P
C24	<1	<1	ND	TNTC	2	0.5	0.05	0.002	<0.05	<0.1	F
C25	<1	<1	ND	TNTC	7.2	0.2	0.05	0.002	<0.05	<0.1	F
C26	<1	<1	ND	TNTC	5.3	1	0.05	0.002	<0.05	<0.1	F
C27 ⁺	<1	0	ND	<1	4	0.6	0.05	0.002	<0.05	<0.1	P



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*UC1	<1	0	ND	TNTC	3.1	0.2	0.05	0.002	<0.05	<0.1	F
*UC2	<1	0	ND	<1	10.9	1	0.05	0.002	<0.05	<0.1	F
*UC3	<1	<1	ND	<1	2.6	0.4	0.05	0.002	<0.05	<0.1	F
UC4 ⁺	<1	<1	ND	1	3.9	0.2	0.05	0.002	<0.05	<0.1	P
*UC5	<1	<1	ND	TNTC	4.5	0.3	0.05	0.002	<0.05	<0.1	F
*UC6	<1	0	ND	<1	4.2	2	0.05	0.002	<0.05	<0.1	F
UC7 ⁺	<1	0	ND	<1	6	0.4	0.05	0.002	<0.05	<0.1	P
UC8 ⁺	<1	0	ND	<1	2.3	0.2	0.05	0.002	<0.05	<0.1	P
*UC9	<1	<1	ND	<1	5.6	0.1	0.05	0.002	<0.05	<0.1	F
*UC10	<1	0	ND	TNTC	2.1	5	0.05	0.002	<0.05	<0.1	F
*UC11 ⁺	<1	0	ND	<1	0.07	0.7	0.05	0.002	<0.05	<0.1	P
*UC12	<1	0	ND	TNTC	<0.07	0.3	0.05	0.002	<0.05	<0.1	F
*UC13	<1	<1	ND	58	4.2	0.4	0.05	0.002	<0.05	<0.1	F
UC14 ⁺	<1	<1	ND	<1	2.1	0.2	0.05	0.002	<0.05	<0.1	P
UC15	<1	0	ND	TNTC	1.9	1	0.05	0.002	<0.05	<0.1	F
UC16 ⁺	<1	0	ND	<1	2	1	0.05	0.002	<0.05	<0.1	P
STD	Absent	Absent	Absent in 25 ml	100 Max. (cfu/ml)	0.5 (Max non- alcoholic) 0.6-15 (Alcoholic)	2 Max.	0.05 Max.	0.003 Max.	0.05 Max.	0.001 Max.	

*STD = Standard, C = certified products, UC = Uncertified product, Staph= Staphylococcus aureus, Max. = Maximum, ND = Not detected in 25 ml, P = Passed, F = Failed. *product was labelled as non-alcoholic. ⁺Sample which passed all the parameters in the specification.*

Table 2. 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



Table 3. Food safety knowledge of Kombucha processors

Food safety knowledge questions/statements	Response (Frequency)	
	Correct	Wrong
Necessary to have your product certified by UNBS	8	0
Hand washing prior to processing can affect Kombucha safety	8	0
Hand washing after touching money can affect Kombucha safety	8	0
Hand washing after using washrooms can affect Kombucha safety	8	0
Hand washing after touching the body can affect Kombucha safety	7	1
Hand washing after using the phone can affect Kombucha safety	6	2
Hand washing after each break can affect Kombucha safety	7	1
Hand washing after handling garbage can affect 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 pathogens	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

Table 4. Self-reported food safety practices of Kombucha processors

Food safety practice questions/statements	Response (Frequency)	
	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 the 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 utensils before processing	7	1
Sanitize utensils after processing	8	0
Sanitize packaging material before use	5	3
The facility is vermin proof storage	8	0
Use treated water for Kombucha processing	8	0
Use of objective methods to test the 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

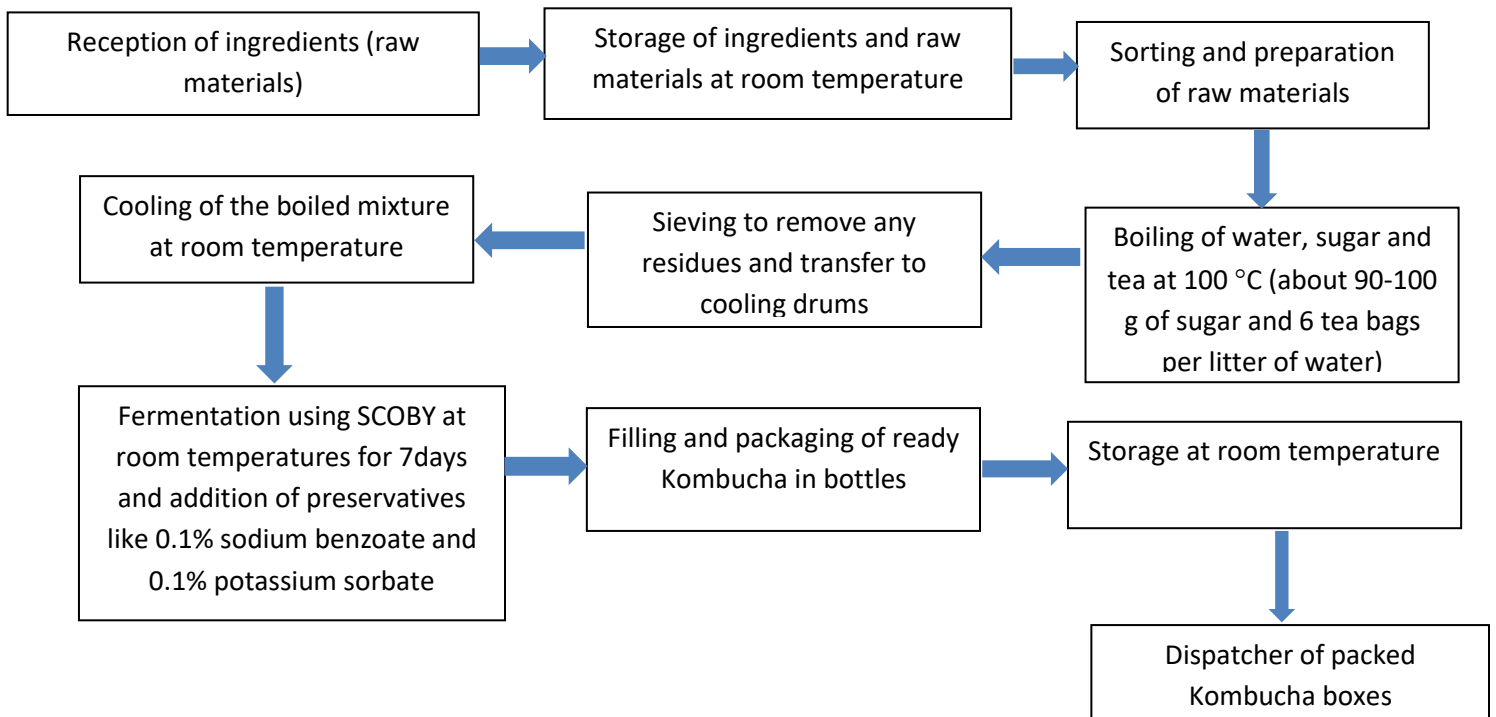


Figure 1. Kombucha flow diagram prior to HACCP plan adoption



Table 5. 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> Qualified raw materials and ingredients Checked Material Safety Data sheet Approved suppliers list Use of specifications 	<ul style="list-style-type: none"> Reject defective batches of supplies 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	<ul style="list-style-type: none"> Proper safety data sheets for raw material quality and storage conditions as per standards Constant monitoring and regular microbial counts checks to ensure safety of raw materials 	Routinely monitoring of the humidity in the stores to prevent wetting of sugar and other raw materials to prevent microbial growth	Proper storage in dry places and humidity checks	Reject the raw material	Humidity log sheets and microbial counts	Assigned Quality assurance Officer
Boiling water and added raw materials such as sugar and tea leaves CCP# 1	Microorganisms (Yeast and molds, <i>E. coli</i> , <i>Staphylococcus aureus</i> and <i>Salmonella</i> spp)	<ul style="list-style-type: none"> Not more than 100 CFU/ml for yeasts and molds Absent in 25ml for <i>Salmonella</i> spp Absent for <i>Staphylococcus aureus</i> Absent for <i>E.coli</i> 	Check the Core Temperature and time	Each batch	<ul style="list-style-type: none"> 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 Core Temperature log; Maintenance register	Assigned Quality Assurance Officer
Sieving CCP# 2	Physical contaminants	No physical foreign matter	Check the sieve clothes for the right	Each Batch	Prior check of sieve clothes for hygiene and right sieve sizes	Changing the sieve clothes to	Inspection report	Production Manager

	(insects, small stones and dust		sizes prior to sieving of the product			replace right ones,		
Fermentation using SCOBY CP# 3	Pathogenic microorganisms	<ul style="list-style-type: none"> No pathogenic microorganism s: <i>Salmonella</i> spp (Absent in 25 ml), <i>Staphylococcus aureus</i> (absent in what volume?) <i>E.coli</i> (absent in what volume?) 	Monitoring the fermentation conditions like temperature and final 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 Pathogenic microorganisms, or utensils and handlers during the fermentation process	<p>Absent for pathogenic molds</p> <ul style="list-style-type: none"> No pathogenic microorganism (Absent in 25ml for <i>Salmonella</i> spp Absent for <i>Staphylococcus aureus</i> <p>Absent for <i>E.coli</i></p>	Check the core temperature (CT) of the product and holding time	Each batch	<ul style="list-style-type: none"> Heating to 85 °C and holding for 10 minutes (Leonarski, et al., 2022) 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 is to be used	Visual inspection for foreign materials, hygiene, leaking, and following of packaging specifications	Each Pack	<ul style="list-style-type: none"> Disinfection of packaging bottles Personal hygiene and physical inspection Use of specifications for packaging materials 	Retain, rework, or discard based on foreign material identified	Inspection report	Packaging operator and Quality Assurance Manager
Storage and distribution of Kombucha CP# 5	Microorganisms from packaging materials)	Absent	Check the time and temperature regime	Routinely	<ul style="list-style-type: none"> Keeping the products at < 4°C for a shelf life 14 days Check storage temperature, shelf life 	Retain or reject based on the product testing panelist Record keeping	Temperature log; Delivery report	Quality assurance Manger



Table 6. Comparison of microbial and physicochemical parameters of Kombucha products pre and post-HACCP implementation

Parameter	Before HACCP implementation		After HACCP implementation	
	Sample	Values	Samples (week)	Values
Microbial				
<i>Escherichia coli</i> (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
<i>Staphylococcus aureus</i> (CFU/ml)	1	<1	1	<1
	2	<1	2	<1
			3	<1
			4	<1
<i>Salmonella</i> 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

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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 a row are significantly different ($P < 0.05$).

