Characterization of a traditionally distilled illicit liquor, ***kachasu,*** in Lusaka, Zambia.

By

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A dissertation submitted in partial fulfillment of the requirements for Master of Science in Food Science and Technology

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We confirm that the work reported in this research report was carried out by the student under our supervision.

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APPROVAL

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DEDICATION

I dedicate this work to my mother, Fungai Simbiso Zizhou, and my late father, Ramios Ruvarashe Zizhou.

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My deepest gratitude goes to the almighty for making a way for me to be able to pursue this study. I would also like to thank my supervisors Dr. H. B. Moonga and Dr. J. Shindano for their guidance throughout the study.

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ABSTRACT

Kachasu is a traditional distilled liquor that is manufactured in Zambia, Zimbabwe, Uganda and the Democratic Republic of Congo. The manufacture and sale of *kachasu* is illegal in Zambia, however this has not hampered its manufacture. *Kachasu* has also been suspected of having a high alcohol content. The objective of this study was to establish the chemical composition, manufacturing processes and the microbes involved in the fermentation of *kachasu*. The study was conducted in Kalingalinga and Ng'ombe compounds in Lusaka, Zambia. The manufacturing process was investigated using a questionnaire. Distillate and mash samples were collected and analyzed. The mash samples were inoculated on selective media and the colony forming units counted. The iron, copper and cadmium contents of the distillate were determined by a Varian Atomic Absorption Spectrophotometer. The primary raw materials were found to be table sugar, maize malt and water. There were seven distinct manufacturing methods identified. The *kachasu* that is produced in Kalingalinga and Ng'ombe compounds of Lusaka was found to have an alcohol percentage ranging from 1.53 to 71.12%. The average alcohol content was 35.03±18.45%. The *kachasu* that is manufactured in the two compounds is collected in three grades commonly known as 'number 1', 'number 2' and 'number 3'. Grade number 1 was found to have a mean alcohol content of 52.06±7.85%, number 2 had 33.68±11.83%, and number 3 had 13.49±9.74%. These differences were significant ($p<0.05$). The retail samples are commonly known as 'mixed samples' and had a mean alcohol content of 23.26±10.10%. The study also found *kachasu* to be an acidic product with a mean pH of 3.79±0.26 for number 1, 3.55±0.302 for number 2, 3.29±0.19 for number 3, and 3.31±0.19 for the retail sample. Four samples tested positive for methanol using the iodoform test out of 40. Some of the distillate samples contained low amounts of copper and iron. However, all the samples had undetectable levels of cadmium. Lactic acid bacteria, cultured yeasts of the *saccharomyces* strains and wild yeast were found to be in the mash during fermentation. The manufacture of *kachasu* is carried out under unsanitary conditions. Some of the *kachasu* produced in Kalingalinga and Ng'ombe had an alcohol content above 40% which is the maximum permissible content according to the Potable Spirits Act of the Republic of Zambia. In conclusion, the *kachasu* being manufactured in Kalingalinga and Ng'ombe compounds of Lusaka, and possibly other areas of Lusaka has a high alcohol content of up to 71.12% and may contain methanol.

Contents

LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of Variance
ET	Ethanol
FID	Flame ionization detector
GC	Gas chromatography
HPLC	High Performance Liquid Chromatography
K	Kalingalinga
LAB	Lactic acid bacteria
LSD	Least significant difference
PEG	Polyethylene glycol
N	Ng'ombe
MRS	De Man-Rogosa-Sharpe
MET	Methanol
NAFDAC	National Agency for Food and Drug Administration and Control
NISIR	National Institute of Scientific and Industrial Research
WHO	World Health Organization

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CHAPTER 1: INTRODUCTION

1.1 Background

Kachasu is an African, traditionally fermented, highly intoxicating, distilled alcoholic spirit that is brewed in the Democratic Republic of Congo (DRC), Kenya, Malawi, Rwanda, Zambia and Zimbabwe (Gadaga et al., 1999). In Malawi, Zambia and Zimbabwe, it is known as *kachasu*, in Kenya as *urugwagwa* (Mkuu et al., 2019a), Rwanda as *kanyanga* (Lyumugabe & Songa, 2019), Nigeria as *kaikai/ogogoro/apeteshi* or illicit gin (Ohimain, 2016) and DRC as *lotoko* (Gadaga et al,1999).

Kachasu is normally brewed from maize. However, other adjuncts like millet (Nyanga et al., 2008a) sorghum, cassava and plantain (Gadaga et al., 1999)and fruits like *masau* (*ziziphus mauritiana*) and banana peel (Nyanga et al., 2008b) can also be used. The manufacturing process relies on spontaneous fermentation in most areas and can also involve addition of brewer's yeast together with the carbohydrate sources to warm water and heating the mixture for a few minutes. The product is then allowed to ferment for several days, depending on the weather conditions. Distillation then follows using makeshift distilleries (Nyanga et al., 2008b). The alcohol (ethanol) content of *kachasu* can vary significantly, depending on the strength of the brew. Lusaka Times (2021) has reported an alcohol content ranging from 20 to 70%. They also claim that its alcohol content is sufficient for use as a biofuel.

Kachasu production and sale is an illegal business in Zambia. There have been calls to legalize its production as it is being produced and sold in high-density populated compounds. Its consumption has been linked to mental illness as reported by the Chainama Hills Mental Hospital in Zambia (Lusaka times, 2021). A report in the African press revealed that patients, mostly youths, as young as 16years, are being treated for mental illness due to alcohol abuse. "In Nigeria, the consumption of *kaikai* was associated with 89 deaths between April and June of 2015. Laboratory analysis that was carried out by the World Health Organization (WHO) and National Agency for Food and Drug Administration and Control (NAFDAC) showed that the beverage contained 16.3% methanol, while the blood methanol concentration of the victims was 1500-2000mg/l (Ohimain, 2016)." "Victims exhibited symptoms of methanol poisoning including loss of consciousness, dizziness, weakness and breathing difficulties, blurred vision and blindness, weight loss, headache, abdominal pains, nausea, diarrhea and vomiting (Ohimain, 2016)." The purpose of this study was to determine the manufacturing process of kachasu, characterize its physico-chemical properties and determine the involvement of lactic acid bacteria, cultured yeast and wild yeast.

1.2 Problem statement

In Zambia, the production and sale of *kachasu* is illegal as highlighted by *The Traditional beer Act Chapter 168 of the laws of Zambia of 1974.* The Act classifies *kachasu* as a traditional beer understandably, as its manufacture is based on traditional knowledge, and it has not been commercialized. However, from its manufacture and appearance, it qualifies to be called a distilled spirit.

In Zambia, distilled spirits are regulated by Statutory Instrument 18 of 2020 – Portable Spirits. This instrument regulates the level of compounds in distilled spirits as stipulated in Table 1.1. To date, the focus has been on the perceived alcohol content and no studies have been conducted to explore its total acidity, volatile acidity, esters, aldehydes, furfurals, fussel oils, isopropanol, butyl alcohol, ash, copper, lead, iron, and cadmium content. Knowing the levels of these compounds will determine if *kachasu* can be purified and bottled.

Several deaths have been linked to *kachasu* consumption. No chemical within *kachasu* has been identified to be the cause of death. The study seeks to determine physico-chemical properties and quantify selected chemical constituents in *kachasu* and if their quantities are beyond safe consumption levels-based standards for distilled spirits. Methanol has also been associated with traditional distilled liquor. The study will determine if any methanol is present in *kachasu* produced in Zambia.

By virtue of *kachasu* being a traditional liquor, the raw materials used for its production and the manufacturing process has not been documented in Zambia. By interviewing the processors, this knowledge can be harnessed and documented to provide insight on any possibilities of contamination. The manufacturing process can then be optimized for commercialization. This can pave way for the construction of a distillery that will buy from the local manufacturers, purify and package the liquor according to the S.I. 18 of 2020.

Establishing a distillery that can buy *kachasu* from the local manufacturers could potentially create a source of legal income for the manufacturers as the need to earn a living is also a driving force in its manufacture. The nation will get revenue from this market through registration of the processors and associated taxes. This revenue can then be used to educate consumers on responsible drinking practices with respect to *kachasu.* The processors can also be educated on the safe handling practices of *kachasu* as alcohol is a highly flammable product.

Table 1.1: Specifications of a distilled potable spirit in Zambia ('SI-18-of-2020_-Portable-Spirits', 2020)

Parameter	**Specification**	**Analytical Method**
Total acidity, % m/v (as tartaric and acetic acid)	0.04	AOAC 945.08
Volatile acidity %m/v (as acetic acid)	0.02	AOAC 945.08
Esters mg/L (as ethyl acetate)	13.5	AOAC 972.07
Aldehydes, mg/L	2.3	AOAC 972.08
Furfurals, mg/L	0.36	AOAC 960.16
Methanol, ppm	20	AOAC 958.04
Fussel oil, mg/L	3.1	AOAC 959.05
Isopropanol and tertiary butyl alcohol	No precipitate shall form	AOAC 935.16
Ash, %m/m	0.041	AOAC 920.48
Copper as Cu, mg/L	2.0	AOAC 967.08
Lead as Pb, mg/L	0.1	ZSISO 6633
Iron as Fe, mg/L	8.0	AOAC 970.12/970.13
Cadmium, ng/L	1.0	ZSISO 6561
Clearness	Clear	
Colour	Characteristic of the type of spirit	
Odour	Characteristic odour	
Taste	Characteristic of the taste	

1.3 Justification of the study

Alcohol misuse contributes substantially to the global morbidity and mortality burden. Worldwide, it is estimated that one fourth of all alcohol consumed is unrecorded (World Health Organization, 2014). In Kenya, homebrew has been linked to several fatalities and hospitalization (Mkuu et al, 2019). In Nigeria, people have died after consuming *kaikai* contaminated with methanol (Methanol institute, 2013). In Zambia, Chainama Hill Mental Hospital has bemoaned mental illness cases linked to

consumption of *kachasu*. *Kachasu* manufacture is rampant in Zambia as evidenced by the continuous reports of illness and/or deaths associated with its consumption (Africa press,2022). This study could serve as a starting point for a scientific understanding of the microbiological and physico-chemical composition of *kachasu* with the aim of providing data that can be used to improve the production process and to eliminate or reduce toxins if found to be present.

If *kachasu* production could be legalized like in Kenya (Mkuu et al, 2019), it would be regulated by the Liquor Licensing Act of Zambia chapter 167 as well as Statutory Instrument 18 of 2020 (the Compulsory Standards- Potable Spirits). This can be advocated for, if the manufacturing method is known as well as its chemical and microbiological compositions. Once regulated, this would imply that consumers can be openly taught on responsible drinking practices as well as hygiene improvement for the manufacturers. *Kachasu* can then be advertised responsibly.

Ethanol, which is thought to be the major constituent of kachasu, has an array of uses including making food additives, as an antifreeze, as an antiseptic, major raw material for gasoline manufacture, a raw material for cosmetic products, and used a medicinal solvent (All uses of, 2021). Purifying the ethanol in kachasu would make it available for these uses thereby reducing the amount that ends up being available for consumption. This would create an alternative source of revenue for the manufacturers.

1.4 Objectives of the study

The objectives of this study were as follows:

Main objective

- To characterize a traditionally distilled liquor, ***kachasu***, produced in Lusaka, Zambia

Specific Objectives

- To investigate the manufacturing process steps of *kachasu*.
- To determine the physico-chemical properties of *kachasu.*
- To determine the microbial load of selected microbes of the mash during *kachasu* production.

CHAPTER 2: LITERATURE REVIEW

2.1 Definition of *Kachasu*

Kachasu is an African traditionally fermented, highly intoxicating, distilled alcoholic spirit that is brewed in Zambia, Zimbabwe, Democratic Republic of Congo (DRC), Kenya, Rwanda and Malawi (Gadaga et al, 1999). In Malawi, Zambia, and Zimbabwe, it is known as *kachasu* (Gadaga et al,1999), in Kenya – *urugwagwa* (Mkuu et al, 2019), Rwanda – *kanyanga* , Uganda it is called *waragi* (Lyumugabe et al, 2019), Nigeria – *kaikai/ogogoro/apeteshi* or illicit gin (Methanol institute,2013) and DRC – *lotoko* or moonshine (Okienczuk, 2023).

In Zambia it is defined as a traditional beer by the *Traditional beer act of 1974*. In Kenya, it is classified under homebrew. It started off as illegal but was later legalized in a bid to control its consumption and to know the extent of consumption to try and establish responsible drinking practices (Mkuu et al. 2019).

2.2 Classification of *kachasu.*

Kachasu is a spirit that is distilled from fermented cereal or fruits (Nyanga et al, 2012). This implies that it can be classified as a gin or vodka. Gin is a liquor distilled from neutral grains such as barley, corn, rye, and wheat and flavored with a variety of botanicals, which vary by brand. Its flavor profile is herbal and dry. The primary flavor that defines gin comes from juniper berries, which impart its signature "piney" aroma and taste ((Egan et al., 1981)).

Kachasu is a potential gin, as it only needs value addition through the inclusion of juniper berries in its manufacture. According to Overproof (2022), when it comes to gin production, distillers begin with a neutral spirit and then add different botanical flavors and ingredients throughout the distillation process. For example, Hendrick's Gin adds caraway, orange fruit, lemon peel, angelica, cubeb pepper, chamomile, elderflower, yarrow, coriander, and orris root to the juniper berry (Overproof, 2022).

On the other hand, vodka has been defined as a spirit distilled from almost anything, and it can be a catch-all category for white spirits that do not fit elsewhere. Neutral grains (rye, corn, wheat, etc.) and potatoes are the most common, though some vodkas are distilled from beets, grapes, and other bases (Egan et al., 1981). Vodka is composed mainly of water and ethanol but sometimes with traces of impurities and flavorings. Traditionally, it is made by distilling liquid from fermented cereal grains and potatoes since it was introduced in Europe in the 1700's. Some modern brands use corn, sugar cane, fruits, honey, and maple sap as the base. Since the 1890s, standard vodkas have been 40% alcohol by volume (ABV) (80 U.S. proof). The European Union has established a minimum alcohol

content of 37.5% for vodka. Vodka in the United States must have a minimum alcohol content of 40% (Diffords', 2023).

2.3 Raw materials

In Zambia, no literature has been found which clearly identifies the raw materials used in the production of kachasu. In the African region, in Zimbabwe, kachasu is made from maize, millet, banana peels and *masau* (*Ziziphus mauritiana*) (Nyanga et al, 2012). In Nigeria, *pito* is made from maize or sorghum (Tamang et al, 2010). In the DRC, *lotoko* is brewed from either cassava, maize or plantains (Okienczuk,2023.). In Kenya common homebrew traditional beverages include *chang'aa (wuruchi or wirgiik*) a distilled spirit made from grains such as millet or bananas, *busaa (molotek*) which is a maize beer; *muratina (kurubu), mnazi* (coconut ale) and *miti in dawa* made of fermented sugar, yeast and herbs (Mkuu et al, 2019). The cereal/adjuncts is the main source of carbohydrates for fermentation to ethanol (Gadaga et al, 1999). The adjuncts provide starch for hydrolysis to simple sugars and fermentation is accomplished by amylolytic molds and yeasts that are found naturally on the raw materials (Tamang et al, 2010).

2.4 Manufacturing process

The manufacturing process of *kachasu* has some similarities in Africa. The manufacturing process of Kanyanga in Rwanda is very similar to *Kachasu* produced in Zimbabwe (Brett et al., 1992; Gadaga et al.,1999) The process relies on natural fermentation, which is an uncontrolled process. Fermentation is initiated by microorganisms that are present from the raw materials, utensils, equipment and the environment. Because the fermentations are spontaneous and uncontrolled, the product microbiota is inconsistent, and the product quality variable (Gadaga et al, 1999).

In Zimbabwe, *kachasu* is made by soaking masau in water and fermentation allowed for 6 -7 days. Distillation is then carried out by heating the drum and cooling the steam, as illustrated in Figure 2.1(Nyanga et al, 2012).

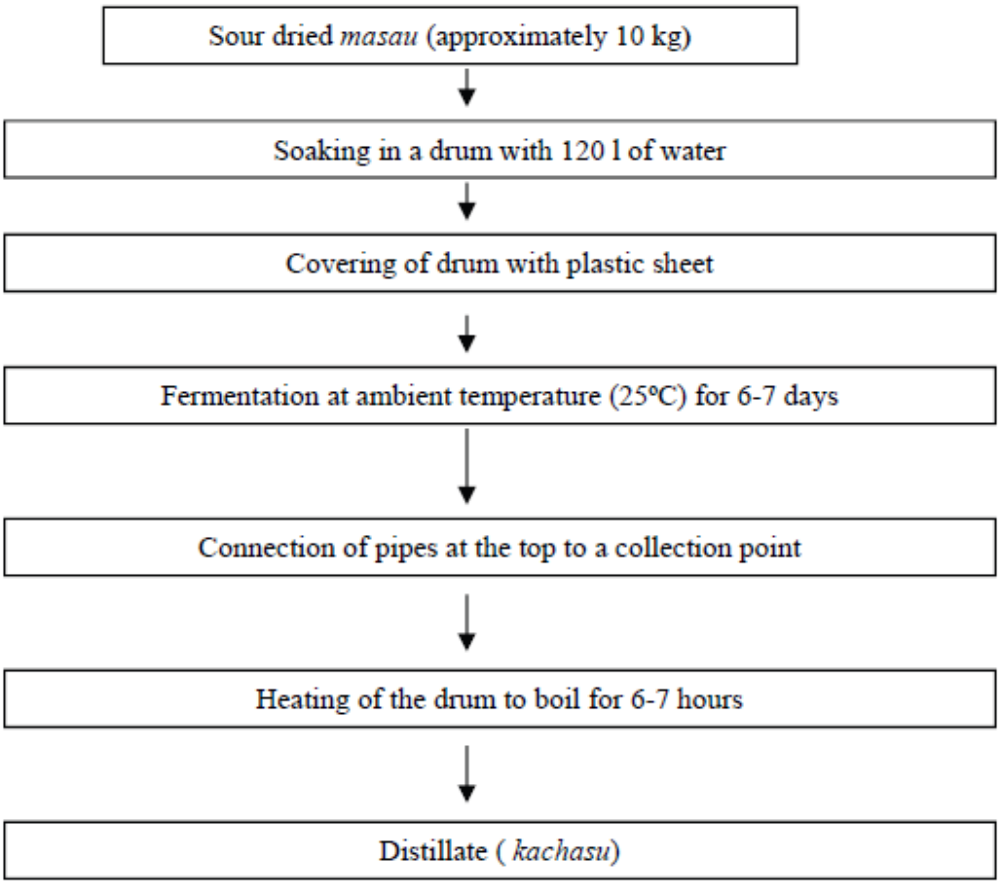


Figure 2.1: Process flow chart of the manufacture of *kachasu* in Muzarabani, Zimbabwe. Source: Nyanga et al, 2012.

In Nigeria, *pito* is an ethnic light brown, alcoholic, sweet-sour beverage made by fermentation of malted, mashed maize or sorghum. Maize is soaked for 2 days, drained and held in a moist chamber for germination/malting for 5 days. The malt is then dried, mashed, and mixed with water. The mixture is boiled for 6-12hours, cooled and filtered. The filtrate is allowed to ferment overnight and boiled for 12 hours to concentrate it. A starter culture is then added which is part of the previous brew. Fermentation is allowed for 12-24 hours (Tamang et al, 2010).

"In Rwanda, *kanyanga is* made by adding raw fermentable materials to warm water in a pot with a hole drilled on the side, which is used later during the distillation of the spirit. The mixture is stirred into slurry and allowed to simmer for a few minutes before the pot is removed from the fire. Sugar and baker's yeast are then added after the slurry has been cooled to ambient temperature. The hole in the pot is sealed with clay and the mixture is allowed to ferment for 4–7 days at ambient temperature. At the end of the fermentation, the seal on the hole is broken and a narrow pipe is connected. The pipe traverses a water jacket containing cold water, which acts as a condenser. The fermented brew is

distilled over a small fire and the clear distillate is collected from the end of the pipe into bottles. Ethanol concentration of k*anyanga* from Rwanda is still not well determined, but it is estimated at 50% (v/v) (Lyumugabe et al, 2022)."

The manufacturing process for *kachasu* in Zambia is not well documented. The literature that gives a bit of insight into the process are mostly newspaper articles and what they show is the distillation process as illustrated in Figure 2.2 B that was extracted from the Lusaka times of 18 May 2022. A study was conducted in Zimbabwe and its focus was on *kachasu* produced from the *masau* fruit and this is illustrated in Figure 2.2 A.

Figure 2.2: A - Manufacture of *kachasu* in Muzarabani, Zimbabwe (Nyanga et al,2012), B-Manufacture of *kachasu* in Zambia (Lusaka times, 2022).

In Zimbabwe, analysis of the ripe *masau* fruits and the fermented fruits during *kachasu* manufacture showed an increase in the yeasts, *Saccharomyces cerevisiae* and *Pichia kudriavevii* colonies and decrease in, *Pichia fabiani. Saccharomycopisis fibuligera and Hanseniaspora opuntiae* were also identified in the fermented pulp (Nyanga et al, 2008). Lactic acid bacteria (LAB) were also found to be coexisting with the yeasts in the fermented pulp. The LAB that were identified in the fermented pulp were *Lactobacilli agilis* and *L. plantarum.* In the fermented pulp, the major organic acids were found to be citric acid and lactic acid. Acetic and malic acid were found in lower amounts. The ethanol composition was found to be ranging from 23.8% to 46.6% (Nyanga et al, 2008). Contaminating yeasts, *Pichia silvicola* and *Pichia anomala* have been identified in the production of a sugarcane beverage called cachaca in Brazil. These yeasts produce 0.5% methanol (Dato et al, 2005). According to the World Health Organization (WHO, 2014) methanol content of 10-220mg/L in distilled spirits is not harmful.

2.5 Food safety

Kachasu is reported to be so strong that it can cause blindness when consumed (Methanol Institute,2013). The consensus from various media houses in Zambia, Malawi and Zimbabwe is that *kachasu* is an illegal product, that has a high alcohol content and results in various medical conditions or even death when consumed (Lusaka times, 2021). Various newspapers have reported deaths after the subjects have consumed *kachasu,* in Zambia as shown in Table 2.1.

Table 2.1: Deaths from consumption of *kachasu*.

Date	Activity	Source
20/03/2023	5-year-old boy in Masaiti district in the Copperbelt dies after drinking *kachasu.*	(‘Africa press, 2022)
31/05/2022	A 27-year-old man of Pojana Village in Kizhinge Zhinge area of Kasempa District has died after consuming a local brew commonly known as *kachasu.*	(Jere, 2022)
25/06/2021	A 40-year-old man of Manyama area in Kalumbila district in North-western province died after consuming allegedly poisoned local beer, *kachasu.*	(‘Lusaka Times, 2021)

In some of the articles (Africa press, 2022), the deaths are thought to be due to the presence of methanol in the traditional distilled beverages. There are different schools of thought about the source of methanol in kachasu. One school of thought is that the methanol contamination emanates from poor hygienic practices during production. The other school of thought is that methanol contamination emanates from the use of substrates rich in pectin. It has been severally reported that microbial fermentation of substrates rich in pectin can result in the formation of methanol (Siragusa et al, 1988; Nakagawa et al, 2000; Mendonca et al, 2011; Ohimain, 2016). Some of the traditional fermented beverages that are prone to methanol contamination were compiled and are illustrated in Table 2.2. Table 2.2 shows that the amount of ethanol in the beverage does not hinder contamination from methanol as it affects both high and low content ones like palm wine which has 40-60% and *burukutu* which has 1.63% (Ohimain,2016). The third school of thought is that contaminating yeast has also been demonstrated to produce methanol during traditional fermentation (Dato et al, 2005). Further, the toxicity of *kachasu* has also been attributed to the presence of several co-generic alcohols such as isoamyl alcohol, isobutanol and methanol. Other organic compounds which have been

identified in *kachasu* include acetaldehyde, acetone, ethyl acetate, and furfurals (Brett et al, 1992). Consumption of these co-generic alcohols in large amounts results in several medical conditions like blindness, and liver cirrhosis. Several compounds could be produced during mixed fermentation with several organisms (Dato et al, 2005).

Table 2.2: Traditionally fermented alcoholic beverages prone to methanol contamination (Ohimain, 2016)

Beverage	Feedstock	Fermenting organism	Countries	Alcohol content
Palm wine	Silver date palm (*Phoenix sylvestris*), the palmyra, jaggery palm (*Caryota urens*), oil palm (*Elaeis guineense*) *Raffia palms*, *kithul* palms, or *nipa* palms. coconut palms *Borassus*	Yeast (*Saccharomyces cerevisiae, Saccharomyces ludwigii, Candida parapsilosis, Candida fermentati, Pichia fermentans, Schizosaccharomyces pombe, Schizosaccharomyces bailli, Kluvyeromyces africanus, Hansenula auvarum, Kloeckera apiculata, Torulaspora delbrueckii*) & Lactic Acid Bacteria (*Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, and *Streptococcus*), acetic acid bacteria (*Acetobacter*, *Aerobacter*)	Most African and Asian countries	
Local gin (ogogoro, kaikai, apetesi)	Palm wine	(*Saccharomyces cerevisiae*) & bacteria (*Lactobacillus*)	Most African and Asian countries	40–60 % Ethanol
Pito (local beer)	Sorghum or maize	Bacteria (*Pediococcus halophilus*, *Lactobacillus*) & yeast (*Saccharomyces cerevisiae, Candida tropicalis, Schizosaccharomyces pombe, Kluvyeromyces africanus, Hansenula anomala, Kloeckera apiculata, Torulaspora delbrueckii*)	West Africa	2–3 % Ethanol
Burukutu	Sorghum	*Sacharomyces cerevisiae, Streptococcus, Lactobacillus, Aspegillus, Fusarium, Penicillium*	Nigeria, Ghana	1.63 % ethanol
Tchapalo (sorghum beer)	Sorghum	Lactic acid bacteria	Cote d'Ivoire	

Beverage	Feedstock	Fermenting organism	Countries	Alcohol content
Tchapalo (sorghum beer)	Sorghum	Lactic acid bacteria (several species)	Cote d'Ivoire	
Bushera	Sorghum	Lactic acid bacteria (several species)	Uganda	0.20–0.75 % ethanol
Ogi	Maize, sorghum or millet	*Sacharomyces cerevisiae, Lactobacillus plantarum, Streptococcus lactis*	Nigeria	?
Urwagwa (banana beer)	Banana		Rwanda	8.7–18 (ethanol), trace (methanol)
Cachaca (banana pulp wine)	Banana	*Sacharomyces cerevisiae*	Brazil	Ethanol (5.34–7.84 %), methanol (0.65–0.189 %)
Cachaca	Sugarcane	*Sacharomyces cerevisiae* and wild yeasts (*Pichia* sp & *Dekkera bruxelensis*)	Brazil	Methanol (0–0.5 %)
Noni	Morinda trifolia	*Lactobacillus plantarum & L. Casei*	Thailand	853 mg/l methanol
Cholai	Rice, sugar-cane, juice of date tree, molasses, and fruit juice (pineapple and jackfruits)	*Sacharomyces cerevisiae*	India	14.5 % alcohol
Dengue	Millet	Lactic acid bacteria (several species)	Burkina Faso	
Kwete	Maize & millet	Lactic acid bacteria	Uganda	

Beverage	Feedstock	Fermenting organism	Countries	Alcohol content
Agave	Agave		Mexico	3.9–339 g/l (ethanol), ND-1826 mg/l (methanol)
Plum wine	Plum		Romania	53–76 % (ethanol), 554–4170 mg/l (ethanol)
Plum brandy	Plum		Macedonia	47–51 % (ethanol), 564–999 mg/l (methanol)
Plum wine	Japanese Plum (*Prunus salicina* Linn)	Yeast	India	175 mg/l Methanol

2.6 Laboratory Analysis

2.6.1 Physico-chemical analysis

To determine the chemical composition of a distilled spirit, gas chromatography can be used. Historically, gas chromatography with polar polyethylene glycol (PEG, wax type) phases is one of the tools used to characterize distilled spirits. PEG phase columns exhibit excellent selectivity for the flavor elements found in distillates, generating useful composite information. The analysis of distilled spirits is quite challenging because of the high levels of water in the samples (40–80%). In addition, the levels of some of the target compounds are in the lower parts per million (ppm) (Lynam et al, 2016) Gas chromatography can detect various organic components as shown in Figure 2.3. This method was used to determine the organic constituents of a Chinese distilled liquor and it was found to contain acetaldehyde, acetone, methanol and the other constituents listed in Figure 2.3.

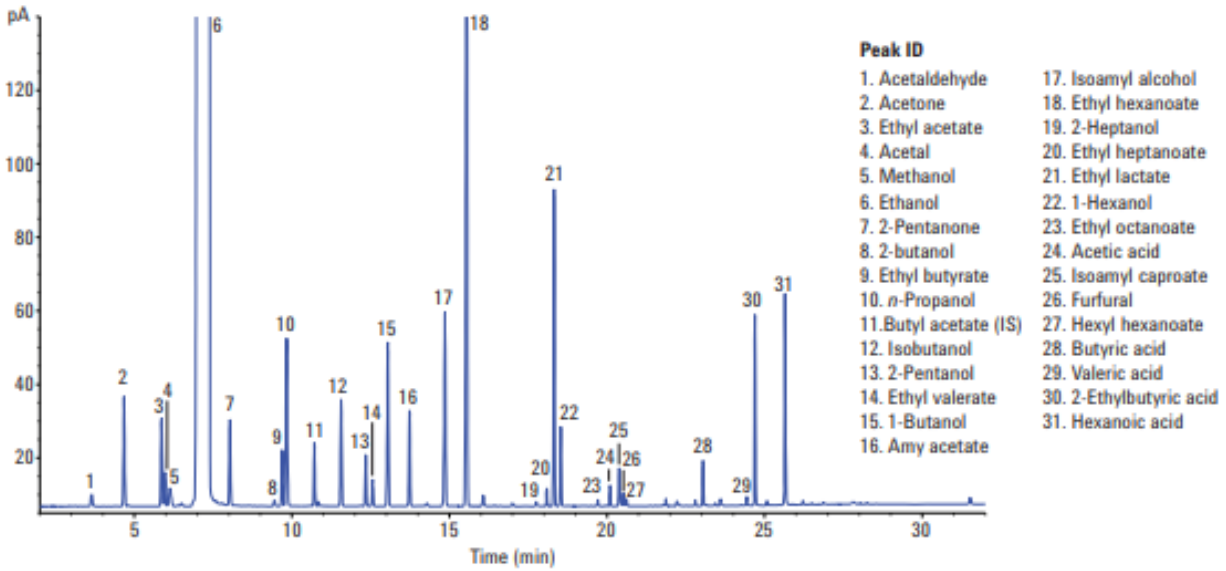


Figure 2.3 Gas Chromatography/Flame Ionisation Detector chromatogram of a Chinese liquor sample using an Agilent J&W DB-WAX Ultra Inert 30 m × 0.25 mm, 0.25 µm GC column (p/n 122-7032UI)(Source: Lynam et al, 2016).

The pH of *kachasu* can be determined using a pH meter with a combination glass electrode and calibrated using buffer at pH4 and pH 7 as most distilled liquors are acidic (Nyanga et al, 2012).

Titratable acidity can be analyzed using a strong base like sodium hydroxide using a phenolphthalein indicator. This is a relatively simple method, that requires a base and indicator. It does not take much time and can be automated. It uses regular chemicals like hydrochloric acid, sodium hydroxide and ammonia. It does not take much time and results are readily available. However, it has the disadvantage that different types of titrations can only be employed within a certain pH range. It is

also a destructive method and may consume large amounts of chemicals (Patil, 2021). This method is also recommended by the Association of Official Analytical Chemists (AOAC) International.

The iodoform test is used for the presence of carbonyl compounds with the structure R-CO-CH_3 or alcohols with the structure R-CH(OH)-CH_3 in each unknown substance. Compounds that give a positive test are acetaldehyde, methyl ketones, ethanol and secondary alcohols that contain methyl groups in alpha position. These compounds result in the production of a pale-yellow precipitate, iodoform, with an antiseptic smell. Methanol is the only primary alcohol that give a negative reaction to this test. The test is relatively simple and gives instant results. However, it is a qualitative test (https://byjus.com/chemistry/iodoform-test/).

2.6.2 Microbiological analysis.

Studies have shown that there is involvement of LAB, cultured yeast and wild yeast in the manufacture of distilled liquor like kachasu (Mkuu et al,2019). Plating samples on selective media has been used to determine the microbial quality of beverages. Buffered peptone water is used for resuscitation of microorganisms and Ringer's water for serial dilutions (Rotar et al, 2012). Serial dilutions enable determination of quantity. Isolation of lactic acid bacteria can be done by plating on DeMann, Rogosa and Sharpe (MRS) agar. *Saccharomyces* yeast grows on Glucose peptone yeast extract agar (Rotar et al, 2012).

Agar can also be referred to as microbiological media, which is a source of nutrients to support the growth of micro-organisms in-vitro. The media helps in the growth and counting of microbial cells, selection of microorganisms, and survival of microorganisms. The culture medium can be liquid or gel. Common ingredients of culture media are peptone (source of carbon and nitrogen), beef extract (source of amino acid, vitamins, minerals), yeast extract (source of vitamin, carbon, nitrogen), distilled water, and agar (solidifying agent) (Fatima, 2022).

Nyanga et al (2012) was able to identify yeasts and lactic acid bacteria coexisting in the fermented pulp that was ready for distilling to *kachasu.* The dominant yeasts were found to be *S. cerevisiae* and *I. orientalis.* The cultured yeasts were enumerated on malt extract agar (MEA) prior to identification. Wort agar can be used to enumerate wild yeast of the *Saccharomyces* genera when incubated at 37°C. Incubation at 30°C enables cultured yeast to grow while suppressing wild yeast (Hutzler et al., 2015).

The dominant LAB found by Nyanga et al (2012) were *L. agilis* and *L. plantarum.* LAB were identified on DeMann, Rogosa and Sharpe (MRS) agar. LAB grows optimally around 30 to 40°C and a pH of 4.4. They ferment glucose to lactic acid if they are homofermentative. Heterofermentative LAB ferment sugars to lactic acid, carbon dioxide and ethanol. Lysine medium

is an excellent agar for enumerating wild yeast. Lysine as the sole source of nitrogen supports the growth of many species of wild yeasts but suppresses the growth of cultured yeast (Lin, 1975).

CHAPTER 3 METHODOLODY

3.1 Study design

A cross-sectional study design with a mixed method was used to conduct this study. The mixed methods involved key informant interviews, a survey of *kachasu* processors and experimental approach. A survey of *kachasu* processors was carried out to determine the processing method(s) to answer specific objective one (1). An experimental study was used to determine the chemical and microbiological characteristics of the in-process and final product to answer specific objectives two (2) and three (3).

3.2 Study site

The study was conducted in Ng'ombe and Kalingalinga which are high density populated compounds in Lusaka. These compounds were purposefully chosen because they are near the University of Zambia and *kachasu* had been reported to be produced and sold there. (Lusaka times, 2021).

3.3. Qualitative Study

3.3.1. Informant Interviews to Identify *Kachasu* Processors

Informant Interviews (KIIs) were conducted to identify key *kachasu* producing compounds in Lusaka. A key informant guide was used for key informant interviews as per appendix 1. The informants were asked about their knowledge on the production of *kachasu* in each respective suburb where *kachasu* is produced and their willingness to introduce the researcher to the *kachasu* producers during the survey. The two key informants that were chosen were respective residents (above 18years of age) of Kalingalinga and Ng'ombe who were willing to facilitate discussions with the producers.

3.3.2 Survey of *Kachasu* Processors

A multi-stage sampling technique was used to sample the City, the compounds and the processors. Lusaka City was purposefully sampled because *kachasu* production had been reported in this city (Lusaka times, 2021). A non-probability sampling technique, purposeful sampling, was used to sample the high-density compounds and *kachasu* processors because a sampling frame was difficult to establish as the production is an illegal business. Further, it was difficult to determine the sample size of *kachasu* processors because the prevalence of *kachasu* processors was not known. The snowball sampling method was used to identify and sample the *kachasu* processors. Two (2) informants identified led the researcher to a few processors they knew, and through snowball

sampling of the *kachasu* processors, a total of 11 processors in Kalingalinga and five (5) in Ng'ombe were surveyed.

3.3.3 Data collection instruments

A survey questionnaire was used to collect data on the processing methods used by different processors (Appendix 2). It focused on the raw materials used, source of the raw materials, raw material storage, the recipes, processing time, processing temperatures, duration per unit operation, fermentation time and parameters used to check for quality of the product. The processors' survey was translated by an Expert in Zambian local languages into Nyanja, a local language mostly spoken in Lusaka, (Appendix 3). It was anticipated that most participants may not be very conversant with the English language. The questionnaires were administered by the researcher.

3.4 Experimental study

The purpose of the experimental study was to determine the physico-chemical composition of *kachasu* produced in Lusaka, Zambia. Further, the purpose was also to determine the involvement of LAB, cultured and wild yeasts in the production of *kachasu* in Zambia.

3.4.1. Baseline Study

A baseline study was conducted in Kalingalinga suburb to check the following:

- To identify *kachasu* processors,
- To check if the environment would be conducive to conduct the research.
- To find out if the processors would be willing to participate in the study,
- To validate the questions for the processors' questionnaire,
- To establish the price of *kachasu* to mobilize funds for sample collection,
- To get an insight on how *kachasu* is made and sold.

Ten (10) processors were identified and interviewed during the exercise. This exercise revealed the raw materials used to manufacture *kachasu*, as well as the price at which it is sold. It also shed light on the fact that the *kachasu* distillate is collected in three parts. The first collection or distillate to come out is called number 1 and this is the strongest (highest alcohol content). The second collection is number 2 and this has a lower alcohol strength compared to number 1. The last collection is number 3 and the volume collected varies from processor to processor. The three stages of collection plus the final retail product, which is called *mixed* were collected. The processors use their experience to collect the different grades. From their recipes, they have determined how much number 1 they get as well as the other two collections. It was also noted that the fermented mixture

of sugar, maize malt and water is called *Matokoso*. This mixture is also referred to as the starter culture for those who carry out backslopping. Samples were bought from the processors at a price range of ZMW 25 – ZMW 50 for a 500ml bottle. The researcher had to blend into the community as shown in Figure 3.1.

Figure 3.1: The researcher with a cooler bag and used 500ml bottles for the processors.

3.4.2. Actual Survey - Sampling of Mash and Distillate

Sampling was carried out at the same time as the administration of *kachasu* processor questionnaire. Virgin, polyethylene terephthalate (PET) bottles and high-density polyethylene (HDPE) closures were used to collect the samples to avoid contamination of the sample by packaging. PET and HDPE have good barrier properties that prevent egress or ingress of chemicals from the environment into the sample and vice versa (Pocock, 2024).

The samples collected from Kalingalinga were denoted by the letter “K” and those from Ng’ombe the letter “N”. They were then assigned numbers from 01 to 11 according to the sequence of collection. After naming the processor with the letter and number, the type of sample was also noted whether it was number 1, number 2, number 3 or mixed for the distillates. The distribution of samples collected is shown in Table 3.1. For the mash samples, the fermentation day was noted as well as whether it was a starter culture.

Table 3.1 Distillate samples collected.

Sampling Site	Number of Distillate samples by collection stage			
	Number 1	Number 2	Number 3	Mixed
Kalingalinga (Baseline study)	9	0	0	1
Kalingalinga	3	3	3	3
Ng'ombe	5	4	4	5

The mash samples were placed into a cooler bag upon collection. They were then frozen at -18°C prior to analysis. The distribution of the mash samples collected is shown in Table 3.2.

Table 3.2 Mash samples collected.

Sampling Site	Number of Mash samples by fermentation day or stage.					
	Starter	*Matokoso*	Day 1	Day 3	Day 4	Day 7
Kalingalinga	3	3	1	3	3	2
Ng'ombe	0	4	0	0	0	1

The mash samples were collected in PET bottles and placed in a cooler bag for transport to a freezer. They were then frozen at -18°C until the time for analysis (Nyanga et al, 2012). The distillate samples were stored at room temperature in PET bottles until analysis since distilled spirits are normally stored at room temperature.

3.4.3. Laboratory Analysis

The distillate and mash samples were analyzed to determine their chemical and microbial composition.

3.4.3.1 Physico-chemical analyses

3.4.3.1.1 pH (Eagan et al, 1981).

The distillate samples were analyzed for pH. 75ml of distillate was placed in a 100ml beaker that had been washed and rinsed with distilled water. An Orion Star A211 pH meter that had been calibrated using Wagtech buffers 4.00 and 7.00 was used to determine the pH. The electrode was placed into the sample and given time to stabilize. A reading was taken when the display showed 'ready'. The electrode was then rinsed with distilled water and dried using a paper towel before immersion into the next sample.

3.4.3.1.2 Total acidity

10ml of distillate were pipetted into a 250ml conical flask as shown in Figure 3.2. Two to three drops of phenolphthalein indicator were added to the flask. The sample was titrated against a standardized 0.1N NaOH until a pale pink color was observed. The total acidity was calculated as follows:

Total acidity as lactic acid = titre value X 0.09 (Eagan et al, 1981).

Figure 3.2: Sample preparation for titration to determine total acidity.

3.4.3.1.3 Brix (Eagan et al, 1981).

A dropper was used to collect the distillate and administer it onto an MT032 refractometer. A reading was taken from the graduated scale and recorded as °Brix.

3.4.3.1.4 Alcohol content (AOAC 982.10).

A 50ml pycnometer was used to determine the specific gravity of the distillate. A Stuart SWB series water bath was switched on and set at 30°C. The pycnometers were first charged with distilled water and allowed to attemperate for 30minutes. Figure 3.3 shows the attemporation of the samples in the water bath. Adjustments were made so that the water would be at the mark by removing or adding water. The samples were allowed to attemperate for another 15minutes, and adjustments made again. The pycnometers were then weighed using an analytical balance (Sartorius Enteris ENTRIS224-1S, Germany) after wiping the outside with a paper towel. After weighing, the pycnometers were emptied and dried in an oven (Blue M brand) set at 105°C. They were cooled in a desiccator and weighed again. The mass was noted. The pycnometers were then charged with the kachasu samples, and the same process repeated minus the weighing of the empty pycnometers. The specific gravity of the distillate samples was calculated as follows.

Specific gravity of sample = (Mass of pycnometer +sample) – mass of pycnometer

(Mass of pycnometer +water) – mass of pycnometer

The alcohol percentage was then extracted from the AOAC tables in section 913.02 Appendix C of the AOAC analytical methods 12th edition, 2000.

Figure 3.3: Attemporation of distillate samples to 30°C in a Stuart water bath.

3.4.3.1.5 Determination of the presence of Methanol

The presence of methanol was determined by addition of 1ml of the sample into a test tube according to Clark (2015). 1 ml of iodine solution was added to the test tube followed by 1ml of 2M sodium hydroxide. The test tube was then placed into a beaker with hot boiled water for 30seconds. The color change was then observed. The presence of methanol was shown by a yellowish color. The presence of ethanol was shown by the development of a white precipitate and the absence of both was shown by the solution becoming colorless as shown in Figure 3.4. The steps were repeated for all samples and the color change noted.

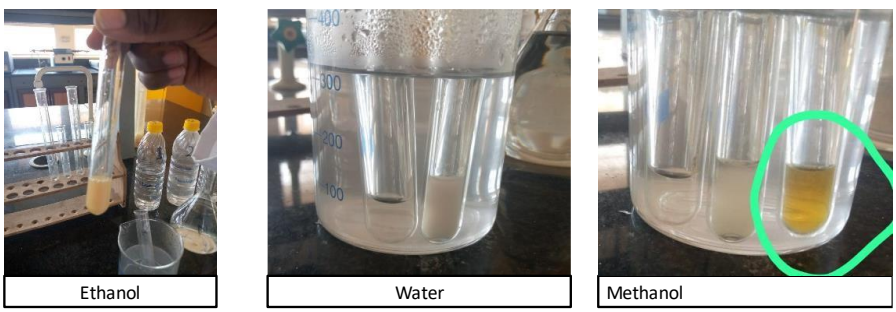


Figure 3.4: Methanol detection using the idoform test.

3.4.3.1.6 Determination of Cadmium (ZSISO 6561), Copper (AOAC967.08), and Iron(970.12).

3.4.3.1.6.1 Sample preparation

10ml of sample were pipetted into a 100ml volumetric flask. Approximately 40ml of distilled water was then added to the volumetric flask and then it was transferred to a fume cupboard. Ten milliliters (10ml) of concentrated hydrochloric acid was then added followed by 10ml of concentrated nitric acid. The flask was then made up to the mark with distilled water. The sample preparation is shown in Figure 3.5.

Figure 3.5: A. Digestion of the mineral samples with concentrated acids in a fume hood and B. Digested samples topped up with distilled water.

3.4.3.1.6.2 Copper Standards preparation

Copper standards solutions were made by dissolving 0.393g $CuSO_4.5H_2O$ analytical grade in a 500ml volumetric flask with distilled water. It was diluted to volume and mixed. This was the working solution 0.004mg/l. 25ml distilled water was added to each of six by 50ml volumetric flasks. 0, 2, 4, 6,8 and 10ml Cu working standard solution was pipetted into each of flasks and diluted nearly to the mark with distilled H_2O. The standards contained 0.0, 0.16, 0.32, 0.48, 0.80, 0.96ppm Cu (µg/ml), respectively. The standards were read at 324.7nm and a standard curve plotted as in Figure a in Appendix 5.

3.4.3.1.6.3 Copper Sample determination

The samples and standards were then read at 324.7nm using a Varian AA280FS Atomic Absorption Spectrophotometer (AAS). The Varian AAS is shown in figure 3.6. A curve was plotted using the

standards and the concentration of copper in the samples calculated from it. The curve is shown in Figure a in Appendix 5.

Figure 3.6: Varian AA280FS AAS used to determine the Fe, Cu and Cd content.

3.4.3.1.6.4 Iron standard preparation.

An amount of 0.0684g of ferrous ethylenediammonium sulfate was added into a 1L volumetric flask. It was dissolved in about 100ml of distilled water. 2.5ml of concentrated sulphuric acid was then added then distilled water topped up and mixed to make a stock solution - 10µg/ml. For the working solutions- 0.0, 0.1, 0.2, 0.3, and 0.4 µg/ml, 0; 1; 2; 3 and 4ml of the stock solution were pipetted into 100ml volumetric flasks containing 50ml 43% alcohol. The solutions were then made up to the mark with 43% alcohol. They were mixed thoroughly, allowed to cool to room temperature, and adjusted to 100ml with 43% alcohol.

3.4.3.1.6.5 Determination of Iron

A Varian AA208FS AAS (Figure 3.6) was used to determine the iron concentration, using lean air-acetylene flame and a single element Fe lamp. The samples and standards were read at a wavelength of 248.3nm. The blank was read first and the AAS was adjusted to 0 *A* while aspirating blank (0.0µg/ml Fe). Distilled water was aspirated between each determination to flush the burner. A standard curve was then plotted of *A* against Fe(µg/ml), and the concentration in µg/ml Fe was determined from this curve shown in Figure a in Appendix 5.

3.4.3.1.6.6 Preparation of Cadmium standards.

1.00g of Cadmium sulphate analytical grade, was dissolved in 10mls of 1:1 nitric acid. The solution was diluted to 1L to give 1000µg/ml of Cadmium (Cd). 0ml, 1ml, 2ml and 3ml of the stock solution were pipetted into 4 100ml volumetric flasks labelled blank, 1, 2 and 3 respectively. The flasks were topped up to 100ml with distilled water. The 4 flasks represented the blank, 1µg/ml Cd, 2µg/ml Cd and 3µg/ml Cd standard solutions.

3.4.3.1.6.7 Determination of Cadmium.

A Varian AA208FS AAS was used to determine the iron concentration, using lean air-acetylene flame and a single element Cd lamp. The samples and standards were read at a wavelength of 228.8nm. The blank was read first and the AAS was adjusted to 0 *A* while aspirating blank (0.0µg/ml Cd). Distilled water was aspirated between each determination to flush the burner. A standard curve was then plotted of *A* against Cd (µg/ml), and the concentration in µg/ml Cd was determined from the graph in Figure a in Appendix 5.

3.4.4 Microbiological analysis (Eagan et al, 1981; Lin, 1975, Adams et al, 2011).

3.4.4.1 Sample preparation.

The mash samples were thawed to room temperature on the laboratory bench while the media was being prepared. After thawing, the samples were diluted using buffered peptone water by adding 1ml of sample into a test-tube with 9ml of peptone water as shown in Figure 3.7. The peptone water was prepared as per manufacturer's (TM Media) instructions and autoclaved at 121°C for 15minutes. The samples were diluted to 10^{-3}.

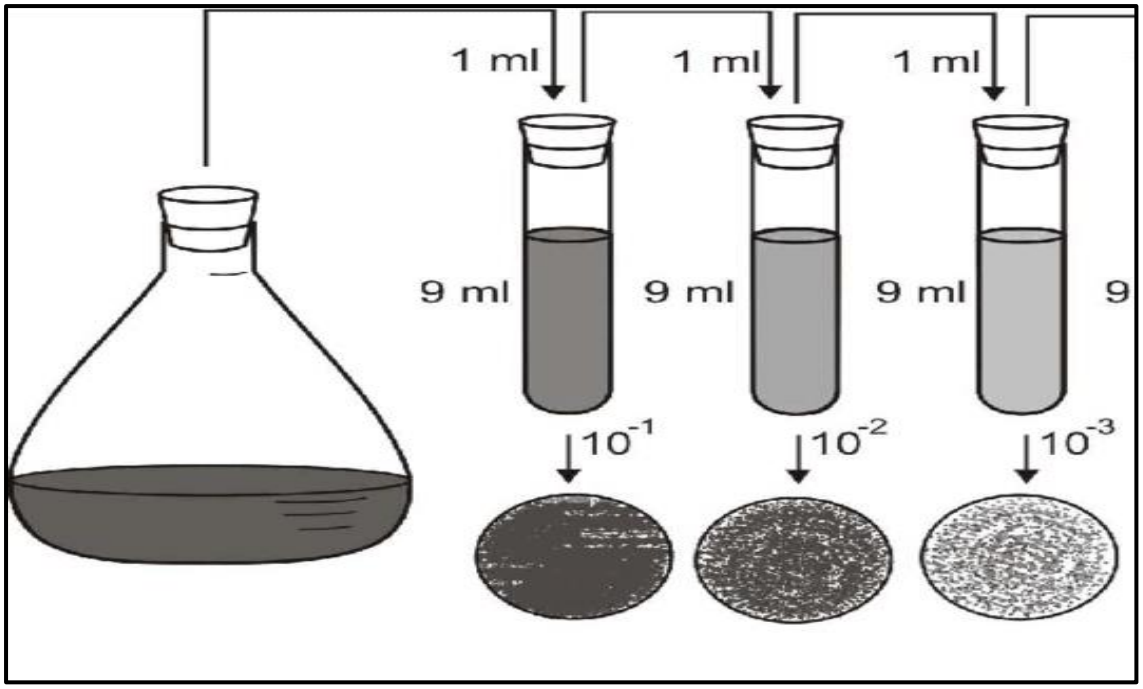


Figure 3.7: Serial dilution of samples

A total of 20 samples were diluted and 1ml of each sample was then pipetted into a petri dish. The petri dishes were labelled with the date, sample number, media and dilution factor. Each dilution factor was pipetted into 3 petri dishes for De Man, Rogosa and Sharpe (MRS), Lysine and Wort agars. Three (3) petri dishes were labelled as controls for the three media types.

3.4.4.2 Determination of Lactic acid bacteria

Eight hundred milliliters (800ml) of De Man, Rogosa and Sharpe (MRS) Agar was prepared as per manufacturer's instructions. It was then autoclaved at 121°C for 15minutes. Samples (1ml) of the diluted samples were inoculated into labelled petri dishes. When the MRS was cooled to around 55°C), it was poured into the 60plates labelled MRS, just enough to cover the base. The plates were swirled and allowed to set in a laminar flow cabinet. The petri dishes were then incubated in a Blue M incubator oven set at 30°C for 48hours. The colonies were then counted using a Fisher Accu-lite colony counter as shown in Figure 3.8.

Figure 3.8: Counting of colonies on plates using a Fisher accu-lite colony counter.

3.4.4.3 Determination of Wild yeast using Lysine agar

The media was prepared according to manufacturer instructions. 10ml of 50% potassium lactate was added to 1L of the media. It was then cooled to 45-50°C. The media was then poured to cover the base of petri dishes labelled Lysine after inoculation with 1ml of diluted sample. The petri dishes were swirled and allowed to set in a laminar flow cabinet. The plates were then incubated at 30°C for 48hours and the colonies read using a Fisher Accu-lite colony counter.

3.4.4.4 Determination of cultured yeast using wort agar

Wort agar was prepared according to manufacturer's instructions. It was then autoclaved at 121°C for 15minutes. While it was cooling, 1ml of diluted sample was inoculated into labelled petri dishes. The media then poured into the petri dishes labelled wort agar. The dishes were swirled and allowed to set in the laminar flow cabinet. The plates were then incubated at 30°C for 48 hours. Colonies were then counted using a Fisher Accu-lite colony counter.

3.5 Data analysis

Qualitative data from the survey was translated into process flow charts to elaborate the processing methods. Quantitative data from the chemical and microbiological analyses were computed using Microsoft Excel. The mean, range and standard deviation were computed for each group. An analysis of variance (ANOVA) was performed on all the groups to determine if there were any

significant differences between their means. The Least Significant Difference (LSD) was then computed where the ANOVA indicated that the means were different at 5% confidence level.

3.6 Ethical considerations

Ethical considerations about the studies were considered. The study involved interviewing the *kachasu* producers to determine the raw materials and process steps for manufacturing *kachasu*. Ethical approval was sought from the Tropical Diseases Research Center (TDRC) Ethics Review Committee under protocol number TDREC/103/02/23 and approval letter number TRC/C4/08/2023 as given in appendix 4. The participants were informed on the purpose of the study, benefits, risks, and the funding behind it. Participation in this study was on a voluntary basis. The processors were identified by the letters K and N for their compounds and a numerical number. K being Kalingalinga and N for Ng'ombe to maintain anonymity. Personal data was not collected. The questionnaire was translated to Nyanja (appendix 3) to make it easier for the processors to understand what was being asked. The interviews were conducted at the premise of the processor, to ensure participant's privacy and confidentiality. The questions asked were limited to the subject on hand, which was the manufacture of *kachasu*. Permission to take photographs of the equipment and raw materials was sought and photographs were only taken where consent was given. No photographs of the processors or their customers or other family members were taken.

CHAPTER 4: RESULTS

In this chapter, the results of the study are presented in line with the specific objectives which were to investigate the manufacturing process steps of *kachasu* (section 4.1); to determine the chemical constituents of *kachasu* (section 4.2); and to determine the microorganisms involved in *kachasu* production (section 4.3). The process starts with the selection and preparation of raw materials.

4.1 Manufacturing process

The manufacture of *kachasu* starts with the selection and preparation of raw materials (section 4.1.1). Processors have different recipes as elaborated in section 4.1.2 and different process steps which are explained in section 4.1.3. They also utilize different types of equipment which is illustrated in section 4.4.

4.1.1Raw materials

The raw materials used for the manufacture of *kachasu* were found to be maize malt, commercially produced white and brown table sugar, water and a starter culture collected from the previous batch of *kachasu*. All the 14 processors used brown sugar except for processor K03 who uses white sugar. Some processors reported that other producers also add commercially produced brewer's yeast purchased from the supermarkets. However, out of all the 14 producers interviewed, none used yeast as they claimed that it affects the taste of the *kachasu*.

Some processors bought dry maize from the market and malted it while others buy ready-made wet malt. The maize was soaked in water in a plastic bucket until it germinated. Thereafter, the germinated maize was spread on the ground on plastic material, chitenge (cloth) fabric, blankets or jute sacks and allowed to continue germinating (Figure 4.1A). Processor K09 retained heat and moisture by covering the germinating maize with an old blanket and routinely sprayed water onto the blanket until the maize germinated to the required growth stage.

Figure 4.1: A. Processor K09 germination of maize on a jute sack and old blanket, and B. Malt drying by processor K10.

The maize is allowed to germinate on the ground close to the household and is vulnerable to flies and animals like dogs as shown in Figure 4.1 B. During the baseline visit to producer K09, it was learnt that sometimes it is even prone to contamination by children as children were observed to be playing with the malt as it was drying. The number of days taken for the malting to be complete was reported to be varied due to the quality of the maize. Some batches were reported to be of good quality and germinated faster whilst others took too long. Overall, it took between 3-5 days.

Malt samples were collected, and these are shown in Figure 4. 2

Figure 4.2: Maize malt samples from some processors: a. shows clean maize malt, b. and c. shows maize malt with molds.

4.1.2 Recipe

All the processors interviewed were found to have their own unique combination of sugar, maize malt, and water. Out of 14 processors, only four (4) processors used the three core ingredients, while the rest added a starter culture called *matokoso* to each batch (Table 4.1). *Matokoso* is either sour mash from the previous batch of *kachasu* or cooled porridge which remains after distillation.

Table 4.1: Recipes of *kachasu* by processor (NB: NS means not stated.)

Processor	Ingredient			
	Sugar (kg)	**Maize malt (L)**	**Water (L)**	***Matokoso (*L)**
K01	30	20	80	20
K03	20	20	110	0
K04	40	20	120	80
K05	60	20	140	20
K06	10	5	60	20
K07	20	20	160	20
K09	50	20	80	40
K10	20	10	80	5/10
K11	25	10	120	0
N01	20	10	30	NS
N02	NS	NS	NS	0
N03	20	10	80	20
N04	10	10	60	0
N05	10	20	NS	NS

Each processor had their own ratio of ingredients. K09 had the highest sugar application rate while K07 had the lowest. Processors N01, N02 and N05 did not fully disclose their recipes as they deemed them to be their trade secrets. Overall, the amount of maize malt added was always less than the amount of sugar added except for K03, K07 and N04 where equal amounts were added and at N05 where more maize malt was added compared to sugar. K10 adds either five (5) liters of *Matokoso* or 10 liters depending on the strength of the sour mash from the *kachasu*.

4.1.3 Manufacturing processes

Fourteen (14) manufacturers were interviewed with nine (9) coming from Kalingalinga (K) and five (5) from Ng'ombe (N) compounds and seven production methods were found (Figure 4.4). The process of making *Kachasu* starts with the malting of maize. Maize kennels are procured from the market and soaked in water for them to absorb moisture. The number of days taken depend on the weather conditions and the quality of maize. After steeping, the maize is allowed to germinate. Additional water may be added. Once the maize has germinated it is referred to as malt. The maize malt is pounded and mixed with water and sugar. This mixture is allowed to ferment spontaneously. Four (4) processors (K01, K05, K07, K11) dry the maize malt prior to pounding.

While mixing, some processors (N05, N01, K04, K06 and K10) also add sour mash to the sugar, maize malt and water mixture. Sour mash is a mixture of maize malt, sugar and water that has fermented and is ready for distillation. This practice of adding sour mash to a new batch is known as backslopping. One processor (N03) takes the cooled porridge from distillation and adds it to the sugar, maize malt, and water mixture. The resultant mixture is then allowed to ferment. Fermentation can take 3 to 7 days depending on the weather conditions. During the hot season or summer, the fermentation period is short (3-4 days) and longer (5-7days) during the winter. Fermentation is carried out indoors. Soon after mixing, the mixture is very sweet. Every day, the mixture is stirred using a traditional wooden cooking stick and tasted for sweetness.

As fermentation of the sugar to alcohol progresses, the sweetness of the mixture reduces due to the depletion of sugar and formation of ethanol, lactic acid, and carbon dioxide ((Egan et al., 1981). When the mixture is no longer sweet and has attained a distinct sourness, it is ready for distillation. Other processors listen to the sound of bubbles that are produced. Soon after mixing, the mixture is quiet due to acclimatization of the microorganisms from the malt and the sour mash to the new environment in a batch. Once acclimatization is achieved, the microorganisms will start multiplying in number. Thereafter, on day 2 the production of bubbles will become vigorous. By day 3, they will be more vigorous and begin to die down towards the end of day 3. On day 4, the mash will be quiet indicating the end of fermentation. When the mash is quiet, then it is ready for distillation. This is the period that it takes for fermentation in the hot season. In winter, the process is longer.

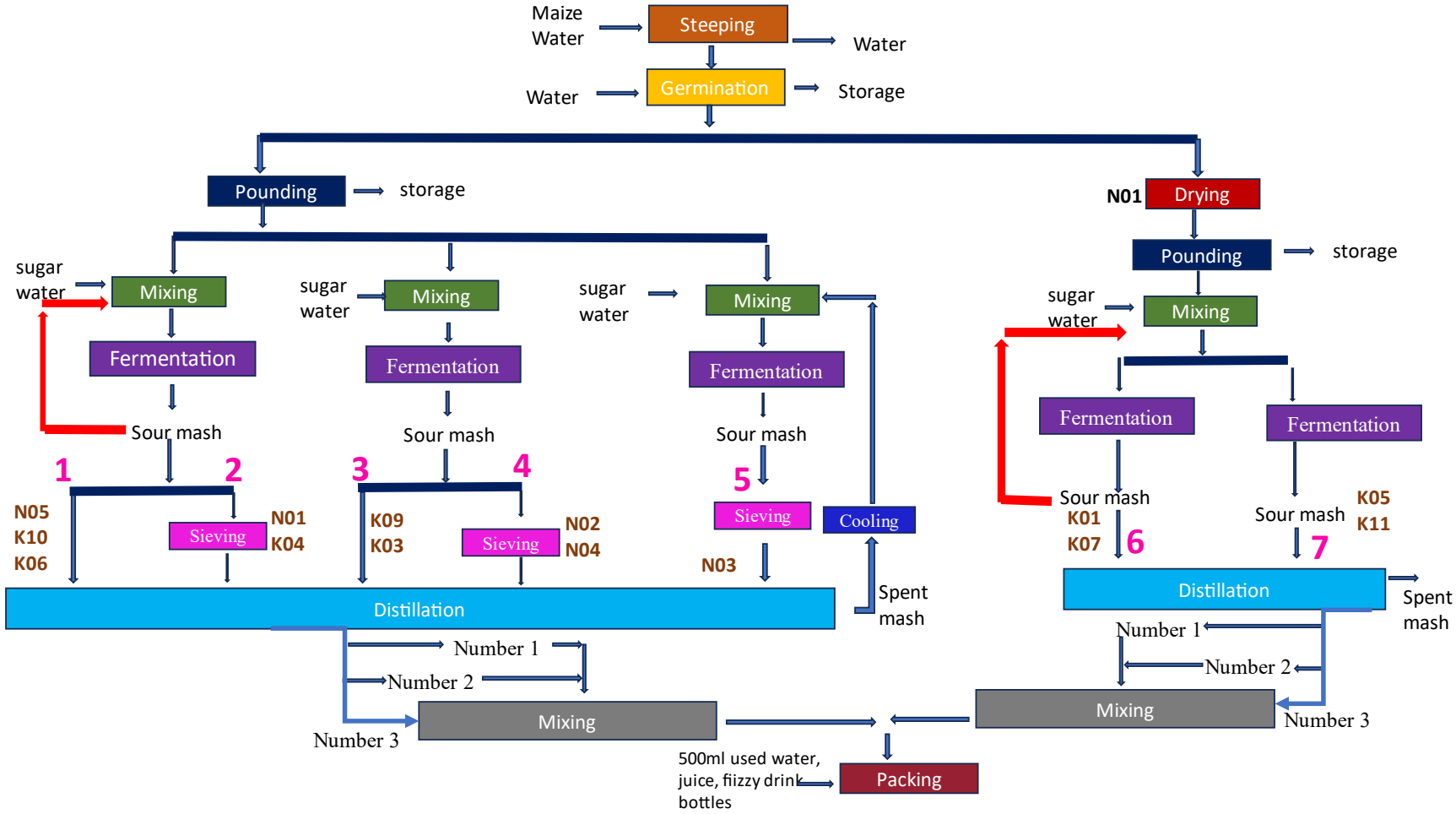


Figure 4.3: The 7 *Kachasu* manufacturing processes that were identified denoted by the numbers 1,2,3,4,5,6 and 7. Each processor is shown by a letter and the processor number (e.g. N01, K11) prior to distillation. Three grades of *kachasu* (number 1, number 2 and number 3) are collected during distillation.

Distillation is carried out during the early hours of the day before the Law enforcement officers start their patrols. The first distillate to be collected is referred to as “number 1”, the second is “number 2” and the last one is termed “number 3”. Each of these constitute three distinct grades of kachasu which are estimated to be approximately 33% each of the total volume collected. Upon sample collection, the number 1 grade was the most expensive. However, the processors normally sell the mixed grade only which is cheaper than number 1 and number 2. Only processor K11 collected number 1 and number 2 simultaneously because of the double barrel designed distillation unit. Each batch is divided into two and fed to a distillation unit as two distillation units are in place. Leftover *nshima/pap/sadza* (stiff porridge) is used to seal the connection between the drum and the condenser pipes. Distillation takes approximately 4 hours. A typical distillation unit is shown in Figure 4.4.

Figure 4.4: Distillation units: A. is double barrel, and B. is single barrel condenser.

After distillation, the *kachasu* is packaged into 500ml bottles. The bottles are pre-used or recycled mineral water, energy drinks and juice bottles. They are washed prior to filling. At processors N01 and K04, the washing process was observed, and it was noted that the water used was very dirty. At processor N01, the washing station was also infested with flies. The source of the bottles was not disclosed. However, while conducting identification of the processors, some people were seen moving around collecting used water and juice bottles from ground litter and from trash bins.

4.1.4 Manufacturing processes identified.

There were seven (7) distinct manufacturing processes that were identified (Figure 4.3). All processing methods start with the malting of maize. Malting is the controlled germination and kilning of a grain. It is a three-step process consisting of steeping, germination and finally kilning (Chaudhary et al., 2014). Some processors omit the kilning (drying) step and use the malt wet (methods 1 to 5 in Table 4.2). All processors pound the malt to reduce its particle size followed by mixing the dry and wet ingredients.

Fermentation is the same across all producers and the duration is determined by the environmental conditions. The differentiation of processing methods comes after fermentation where some processors sieve the mash prior to distillation. Others (N05, K10, K06, K03, K09, K01, K07, K05 and K11) just take the fermented mash and distill without sieving. Figure 4.5 "A" shows the spent grain removed prior to distillation.

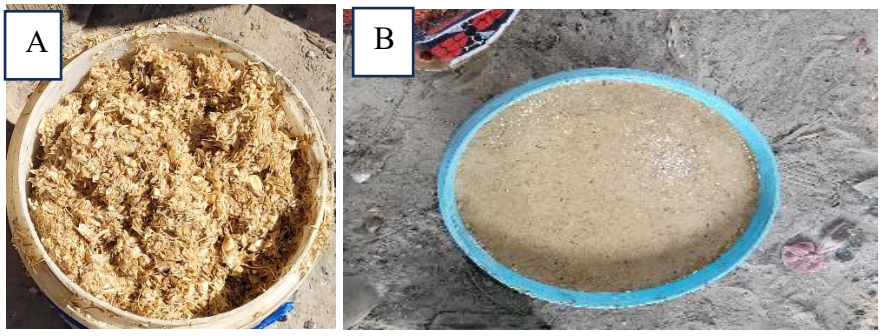


Figure 4.5: A. Spent grain sieved prior to distillation and B. porridge being cooled after distillation.

Methods 2, 4 and 5 have the sieving step as part of the process. The processors highlighted that removal of spent grain is done to avoid burning of the porridge during distillation. Distillation, mixing and packing is the same across all producers. Methods 1, 2, 5 and 6 have backslopping as part of the manufacturing process. In methods 1, 2 and 6, sour mash that is ready for distillation is used for backslopping while method 5 uses the porridge that is left over from distillation. This porridge is cooled first before mixing with all the other ingredients.

Table 4.2: Summary of manufacturing processes

		Kachasu Processing Methods						
		1	2	3	4	5	6	7
Processor		N05, K06, K10	N01, K04	K03, K09	N02, N04	N03	K01, K07	K05, K11
Steeping								
Germination								
Drying								
Pounding								
Mixing								
Fermentation								
Sieving								
Distillation								
Mixing								
Packing								
Backslopping	Sour mash							
	Porridge							

NB: Green indicates a step in the process and black means that step is not part of the process.

4.1.5 Manufacturing Equipment

The processors use similar equipment for manufacturing *kachasu*. A traditional pestle and mortar were used to pound the malt as shown in Figure 4.6.

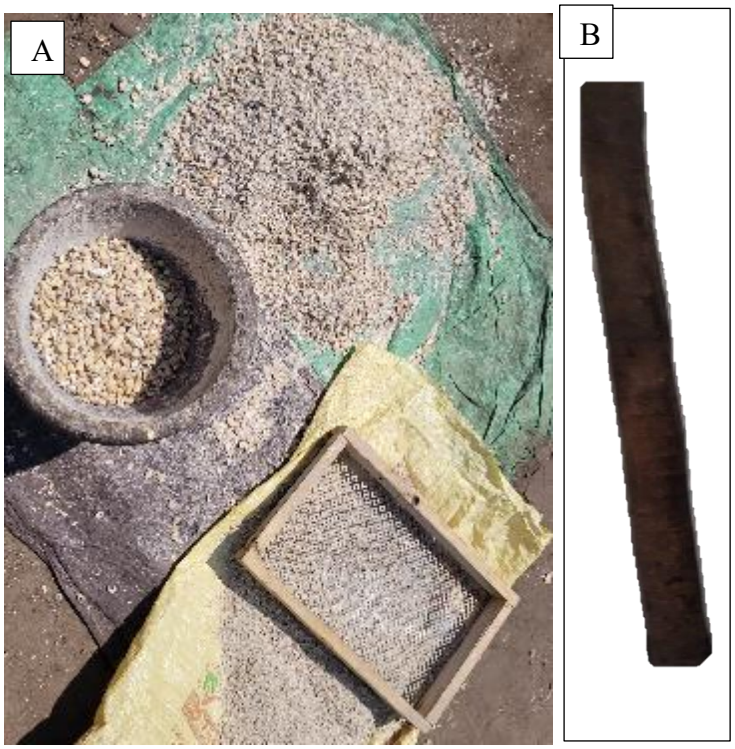


Figure 4.6: A. Mortar used for pounding malt and the sieve. B. Pestle for pounding.

Secondhand buckets (Figure 4.7 A) are used for measuring the maize malt, water, and starter culture. The mash is fermented in blue, 200l plastic drums as per Figure 4.7 B.

Figure 4.7: A. Bucket used for measuring maize malt. B. Plastic drum used as a fermentation vessel.

Distillation is carried out using a 200l metal drum that is fitted with metal pipes (Figure 4.8 A). The metal drum is covered using a black polythene sheet as shown in Figure 4.8 A.

Figure 4.8: A. Distillation drum covered by a black polyethene sheet and secured with a rubber rope; B illustrates the condenser.

The sheet is secured using rubber bands from old bicycle tubes. In some cases, 1 or 2 pipes are used as shown in Figure 4.9 A and B. The pipes are fitted in a dish and water is poured into the dish to condense the distillate. Mud or leftover pap/nshima/sadza is used to seal the connections. The condenser is shown in Figure 4.8B.

Figure 4.9: A. Double barrel condenser and B. Single barrel condenser.

The dish is set on some stones/chair/bucket/another drum. Collection of the distillate is carried out in any of 5l used motor oil bottles (Figure 4.9 B), 5l high density polyethylene (HDPE) natural bottles (Figure 4.9 A), and HDPE buckets(Figure 4.10A). Some processors have funnels for directing the distillate as in Figure 4.9 A, yet some use pre-used water sachets or plastic bags as in Figure 4.10 A. Others just collect directly from the condenser (Figure 4.10 B)

Figure 4.10: A. Water sachets funnels. B. No funnel to direct distillate and collection in a bucket.

Stirring is done using the traditional wooden cooking stick as shown in Figure 4.7 A. The distillation apparatus is fired by firewood (Figure 4.10 A and B) and charcoal.

The hygiene of the processing equipment was below average for all the processors. The blue drums used for fermentation were dirty. Processor N01 washed the 500ml bottles prior to filling. However, the water that was being used for washing the bottles was dirty and there were flies around the surroundings as shown in Figure 4.11.

Figure 4.11: Bottle washing station at processor N01.

4.2 Chemical analysis results

The *kachasu* distillate samples were analysed to determine its chemical properties and constituents. The analysis was carried out at the National Institute of Scientific and Industrial Research (NISIR), Food Science and Nutrition Laboratory in Lusaka, Zambia. The samples were analysed for pH, total

acidity, alcohol content, methanol presence, brix, iron, copper, and cadmium. Section 4.2.1 shows the results of analysis from the baseline study and section 4.2.2 are results from the survey.

4.2.1 Baseline study

The baseline study had nine (9) number 1 samples and one (1) mixed-retail sample. The samples collected during the baseline exercise were analysed and the results are shown in the Table 4.3.

Table 4.3: Baseline study (pH, total acidity, alcohol, brix and iron).

Processor	**pH**	**Total acidity as lactic acid (%, m/v)**	**Alcohol (%)**	**Brix (°)**	**Fe (µg/ml)**
Kalingalinga 1: No 1	3.7	0.09	53.80	15.8	ND
Kalingalinga 2: Mixed	3.36	0.23	22.12	7.4	ND
Kalingalinga 3:No 1	3.52	0.05	24.97	8.8	ND
Kalingalinga 4: No 1A	3.91	0.06	69.07	16.8	0.151
Kalingalinga 5:No 1	3.72	0.13	47.55	14.8	0.017
Kalingalinga 6:No 1A	3.63	0.06	55.49	14.4	0.087
Kalingalinga 7: No 1	3.31	0.11	28.49	9.2	ND
Kalingalinga 8: No 1	4.28	0.03	71.12	17.4	0.082
Kalingalinga 9:No 1	3.6	0.05	48.79	14.8	0.056
Mean	**3.62 ± 0.26**	**0.10 ± 0.06**	**46.85 ± 17.12**	**13.4 ± 3.6**	**0.04 ± 0.05**
Range	**3.31 – 4.28**	**0.03 – 0.23**	**22.12 – 71.12**	**7.4 – 17.4**	**ND – 0.15**

NB: ND stands for non-detected.

The pH ranged between 3.31 and 4.28. The alcohol content varied from 22.1% to 71.12%. Total acidity was 0.03-0.23, calculated as lactic acid. The iron content was 0.00 to 0.015µg/l. The Brix ranged from 7.4 to 17.4°Brix. The copper and cadmium in the baseline samples were non-detectable.

4.2.2 Survey results

During the survey, samples were collected from four (4) processors in Ng'ombe and three (3) processors in Kalingalinga. Each of them produces three (3) grades of *kachasu* number 1, number 2, and number 3 which are then used to make the retail sample that is called 'mixed'. The results are grouped according to the parameters analysed.

4.2.2.1 pH

Number 1 samples had a pH range of 3.45 to 4.13, and the average pH was 3.79 ±0.26. Number 2 samples ranged from 3.33 to 4.21, the mean was 3.55±0.30. Number 3 samples had a lower range of 3.11 to 3.69. The mixed retail sample had a range of 3.11 to 3.45 which was the lowest, and an average of 3.31±0.11. The pH distribution is shown in Figure 4.12 and the detailed table is found in Appendix 6.

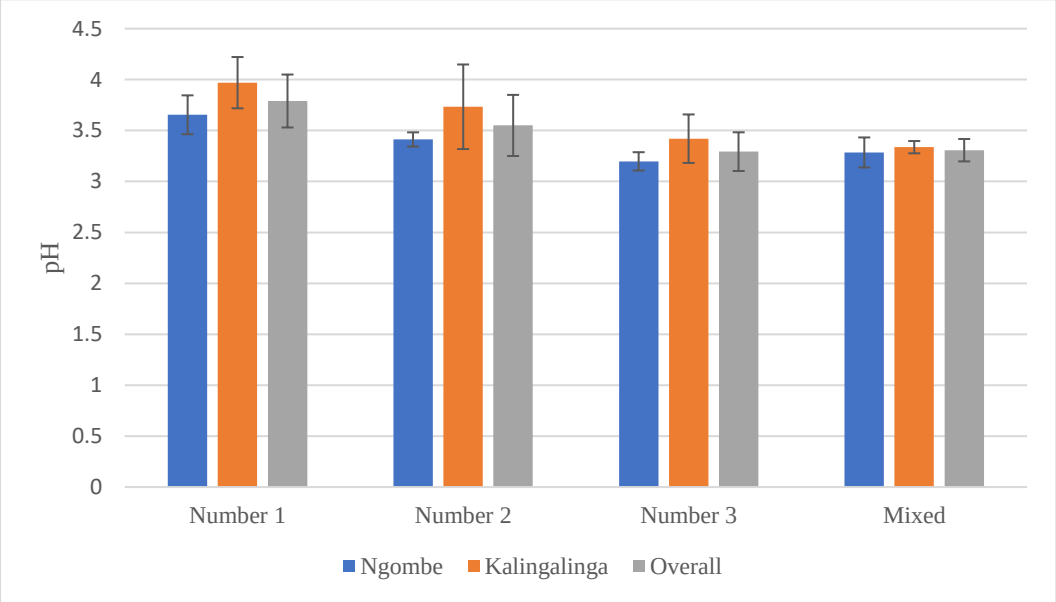


Figure 4.12: pH distribution for *kachasu* distillate by grade and by area.

An analysis of variance (ANOVA) was carried out between the four groups of samples i.e number 1, number 2, number 3 and the mixed sample. F (7.480) ≥ F critical (3.009) at 0.05 level, hence H_0 was rejected leading to the conclusion that there was a difference between the means of the four groups. The LSD was computed, and a significant difference was noted between number 1 and number 3, and number 1 and mixed. Number 1 had a higher pH (3.79) than all the other distillates, but the difference was not significant with number 2 (3.55). The detailed ANOVA results and the LSD figures are in appendix 7.

An analysis of variance was carried out to determine whether the samples from Ng'ombe and Kalingalinga were similar. F value (1.66) was found to be less than F critical (5.99) indicating that there was no significant difference between pH of the two areas. The detailed ANOVA is found in Appendix 7.

4.2.3 Brix

The brix was found to be an average of 14.37± 3.07 for number 1, 9.8 ±3.4 for number 2, 4.9± 4.0 for number 3, and 5.66 ±2.9 for the mixed sample. The distribution is shown in Figure 4.13 and the detailed table is found in Appendix 6.

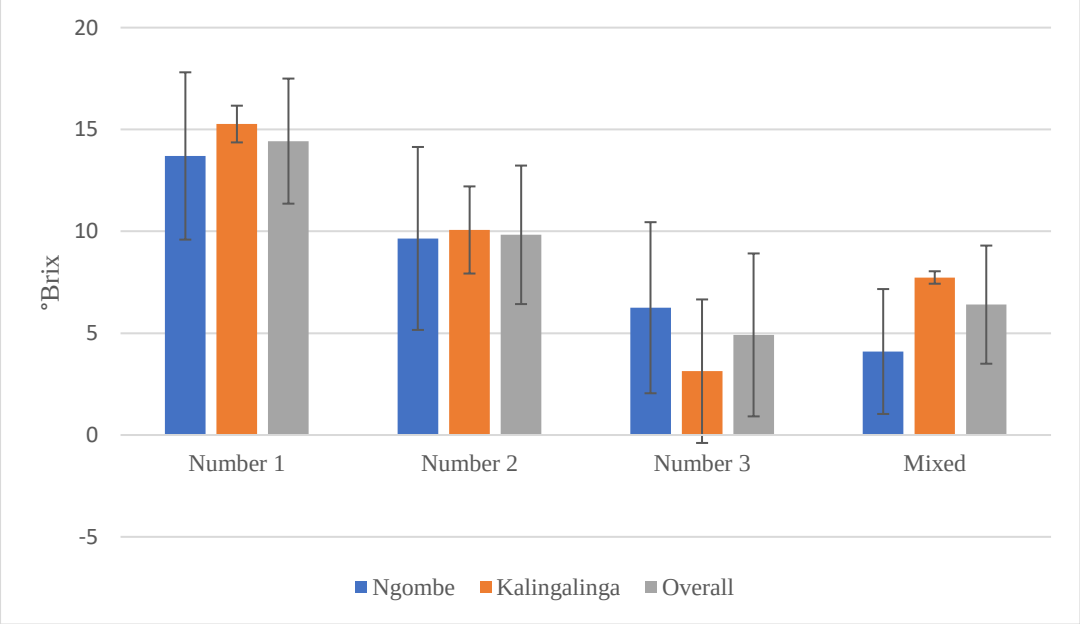


Figure 4.13: Brix distribution for *kachasu* distillate between Ng'ombe and Kalingalinga.

An ANOVA was performed on the four groups of the *kachasu,* and F (10.812) was greater than F critical (3.009), hence H_0 was rejected implying that there was a difference between the means of the four groups. The LSD at 5% confidence indicated that there was a significant difference between all the means. This implies that all the four collections were different in terms of their brix content. The detailed ANOVA and LSD results are in appendix 7.

An ANOVA was carried out to determine if there was a difference between the brix of Ng'ombe and that of Kalingalinga. The F value (0.036) was found to be less than F critical (5.99) indicating that there was no significant difference between the brix found in Ng'ombe and that found in Kalingalinga. The ANOVA table is found in appendix 7.

4.2.4 Total acidity

The mean acidity as lactic acid for number 1 was 0.07 ±0.02 % volume per volume(%v/v), number 2 0.09 ±0.04 %v/v, number 3 0.13 ±0.06 %v/v and mixed was 0.13 ± 0.06%v/v. The acidity of number 1 ranged from 0.05 to 0.10 % v/v. The minimum for number 2, number 3 and mixed were all 0.02%v/v yet the maximum was 0.14, 0.19 and 0.27 respectively. The total acidity distribution is shown in Figure 4.14 and the detailed table is found in Appendix 6.

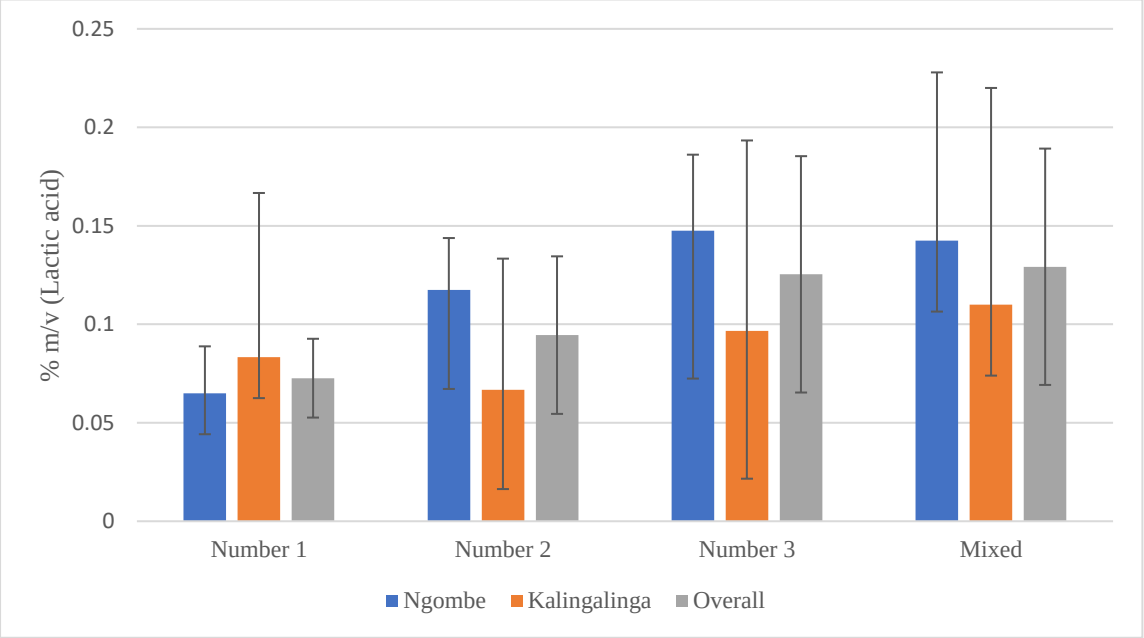


Figure 4.14: Total acidity of *kachasu* distillate by grade and by area.

An ANOVA was carried out and the F (1.9526) was less than F critical (3.009). Therefore, the null hypothesis was accepted and concluded that there was no significant different between the means of number 1, number 2, number 3 and the mixed sample. This implies that the four collections had the same acidity. The ANOVA is in appendix 7.

An ANOVA was carried out to determine if there was a difference between the total acidity in Ng'ombe and that in Kalingalinga. F value (1.895) was found to be less than F critical (5.987) hence there was no significant difference of the brix from Ng'ombe compared to that of Kalingalinga. The detailed ANOVA is found in Appendix 7.

4.2.5 Alcohol content

The average alcohol content was 53.05±7.93% for number 1, 33.68± 11.83% for number 2, 13.49± 9.74% for number 3, and 22.82±10.83% for the mixed one. The amount of alcohol in the samples also had a wide range with number 1 ranging from 44.66 to 65.15% for number 1, 18.49 to 54.85%

for number 2, 1.53 to 24.86 for number 3 and 7.70 to 36.87% for the mixed samples. The distribution of the alcohol content is shown in Figure 4.15 and the detailed table is found in Appendix 6.

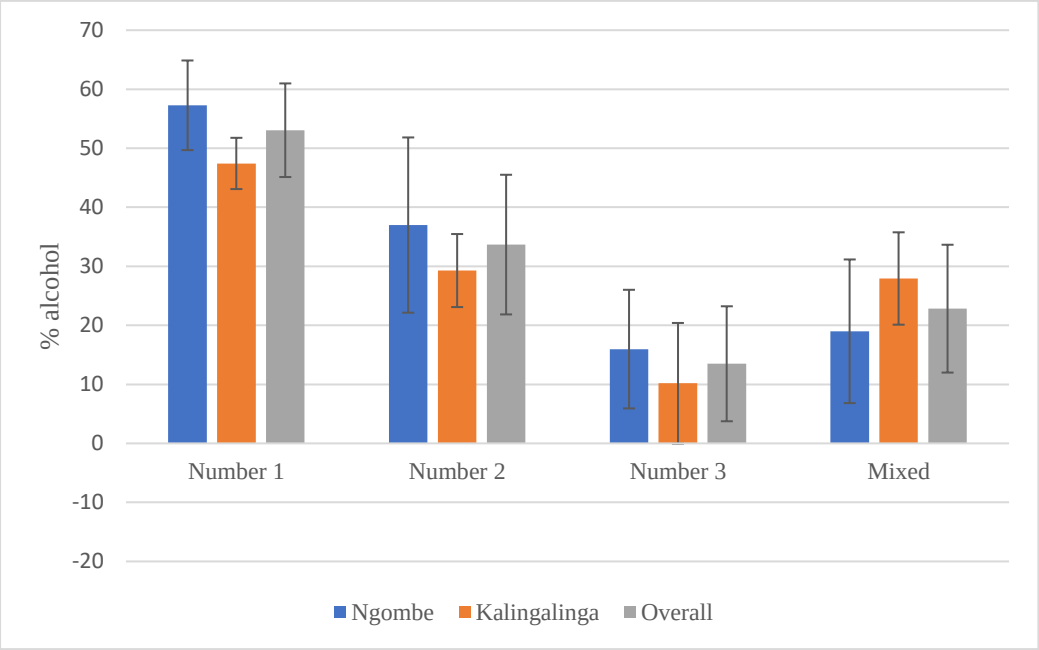


Figure 4.15: Alcohol content of the *kachasu* distillate by grade and by area.

An ANOVA was conducted, and F (19.496) was greater than F critical (3.009) meaning we reject the null hypothesis that the means are equal and conclude that the means of the four groups are different. A computation of the LSD showed that there was no significant difference between number 2 and the mixed samples only. This means that number 1 has a significantly higher alcohol content than all the other collections. The detailed table is in appendix 7.

An ANOVA was carried out and F value (0.087) was less than F critical (5.987) hence there is no significant difference between the alcohol content in Ng'ombe and Kalingalinga.

A correlation analysis was done for the added sugar concentration (kg/l) and the alcohol (%) content of the number 1 grade. The correlation coefficient was found to be 0.4354 indicating an intermediate positive correlation. The graph is shown in Figure 4.16.

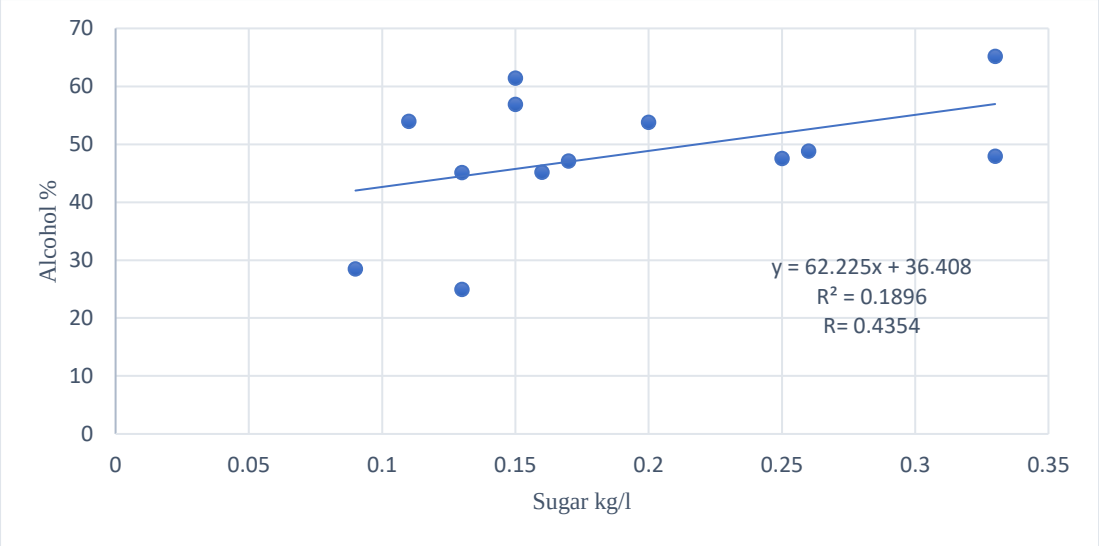


Figure 4.16: Correlation analysis of added sugar concentration (kg/l) and alcohol content (% alcohol).

4.2.6 Methanol test

The iodoform test is a qualitative test that was carried out on all the 40 *kachasu* distillate samples. Only four (4) samples (N03: number 3; N05: mixed; K04: mixed and K06: number 3) tested positive for methanol in the iodoform test out of 40 *kachasu* distillate samples. The reactions of ethanol, methanol and water are shown in Figure 4.17. These four (4) samples were also the ones with the least alcohol content. The other 36 samples tested positive for ethanol by giving the white precipitate.

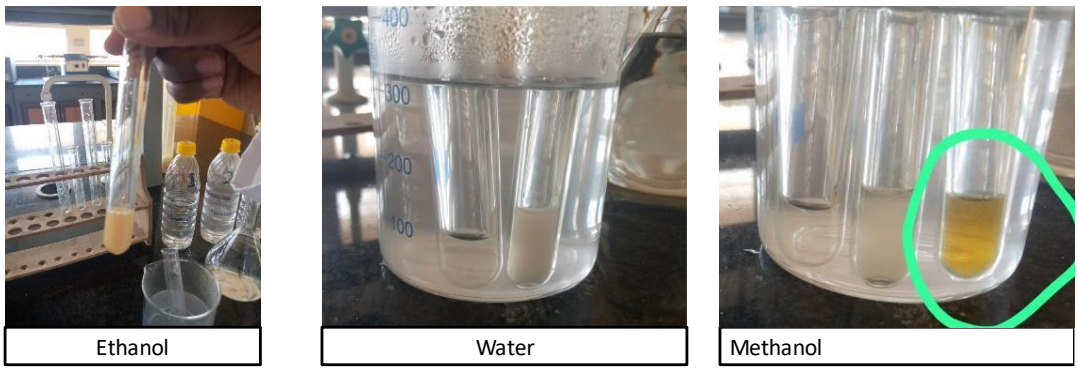


Figure 4.17: Color changes for the iodoform test for methanol.

The detailed results are illustrated in Table 4.4.

Table 4.4: Iodoform test results

Sample	Number 1		Number 2		Number 3		Mixed	
	ET	**MET**	**ET**	**MET**	**ET**	**MET**	**ET**	**MET**
Ng'ombe 1	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Ng'ombe 2	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Ng'ombe 3	+ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve
Ng'ombe 5	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve
Kalingalinga 4	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve
Kalingalinga 6	+ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve
Kalingalinga 11	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve

NB: ET is ethanol and MET is methanol and +ve is positive and -ve means negative.

4.2.7 Mineral contents

The *kachasu* samples were tested for iron, copper, and cadmium. All the samples were undetectable for cadmium. Number 2 samples were all undetectable for iron. The iron concentration at distillation collection stage is shown in Table 4.5.

Table 4.5: Iron content (µg/ml) for *kachasu* by distillation collection stage.

Area	Number 1	Number 2	Number 3	Mixed
Ng'ombe mean	0.079 ±0.059	0±0	0.0582 ±0.0854	0.0245 ±0.049
Kalingalinga mean	0.062±0.048	0	0.0383±0.066	0.024±0.026
Overall mean	**0.07 ±0.05^{a}**	**-^{bc}**	**0.05 ±0.07^{ab}**	**0.02 ±0.04^{c}**
Overall range	**0.02 – 0.158**	**-**	**ND – 0.181**	**ND – 0.098**

NB: ND means non-detected. Means with the same letters not significantly different according to LSD at 0.05 level.

An ANOVA was carried out and F (2.948) was less than F critical (3.009) hence the null hypothesis was not rejected meaning that there was no significant difference between the means of the four groups. Statistically there is no significant difference, but number 2 samples were all undetectable for iron.

An ANOVA was carried out to determine if there was a significant difference of the iron content found in Ng'ombe and that found in Kalingalinga. F value (0.1798) was found to be less than F critical (5.99) hence there was no significant difference between the two areas.

The samples were also analysed for their copper concentration. Five samples had detectable amounts. The distribution of the copper content for the *kachasu* distillate is shown in Figure4.19. An ANOVA was carried out and F (0.042) was found to be less than F critical (3.009) meaning that there was no significant difference between the means of the four distillation collection stages. Statistically the amounts found in the 5 samples were not significant. The distribution of the copper content in the *kachasu* distillate is shown in Table 4.6.

Table 4.6: Copper content (µg/ml) for the distillate (*kachasu).*

Area	**Number 1**	**Number 2**	**Number 3**	**Mixed**
Ng'ombe mean	0.0027 ±0.005	0.00375±0.005	0.00275±0.006	0.0025±0.005
Kalingalinga mean	0	0	0	0
Overall mean	**0.002 ±0.004[a]**	**0.002 ±0.004[a]**	**0.002 ±0.004[a]**	**0.001 ±0.004[a]**
Overall range	**ND – 0.01**	**ND – 0.01**	**ND -0.01**	**ND- 0.01**

NB: Means with the same letters not significantly different according to LSD at 0.05 level.

Only two manufacturers (Ng'ombe 01 and Ng'ombe 02) had detectable amounts for copper. An ANOVA was carried out to determine if there was a significant difference between the copper content in samples from Ng'ombe and that of Kalingalinga. The F value (112.322) was found to be greater than F critical (5.987) hence the copper content for samples from Ng'ombe is different from that of samples from Kalingalinga. The detailed ANOVA is shown in Appendix 7.

4.3 Microbiological analysis

The mash samples that were collected during the baseline study and survey were all inoculated and cultured for LAB, cultured yeast and wild yeast. Figure 4.12 illustrates the outcome of the cultured plates. Some LAB plates had mold growth on them.

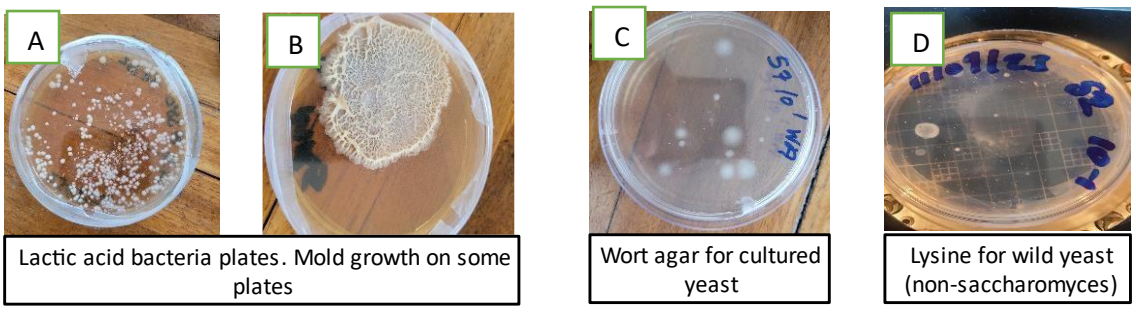


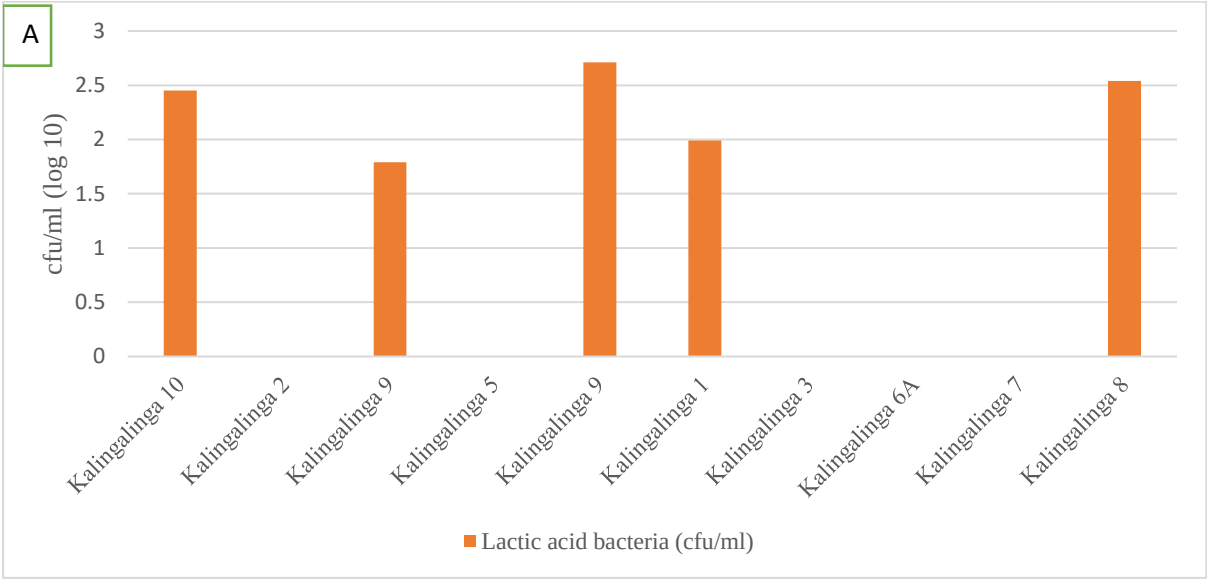
Figure 4.18: Plates showing the culture of: A. lactobacilli, B. mold growth on MRS plates, C. wild yeasts and D. cultured yeast.

4.3.1 Baseline study

The mash samples that were collected and inoculated to enumerate lactic acid bacteria, wild yeasts, and cultured yeast were at different stages of fermentation. The results for the baseline study are shown in detail in Appendix 6. in colony forming units per milliliter of sample (cfu/ml).

The mash samples that were collected were at different stages of fermentation. There was only 1 sample for day 1 mash and it had LAB, cultured yeast, and wild yeast. Five (5) samples had LAB, 6 samples had cultured yeast of the *Saccharomyces cerevisiae strain* and 9 samples had wild yeasts. K02 was not detectable for any of the 3 microorganisms. K09 had the highest LAB count and cultured yeast count, while K05 had the highest wild yeast count.

The plate counts were converted into base 10 logarithmic values and the distribution is shown in Figure 4.19.



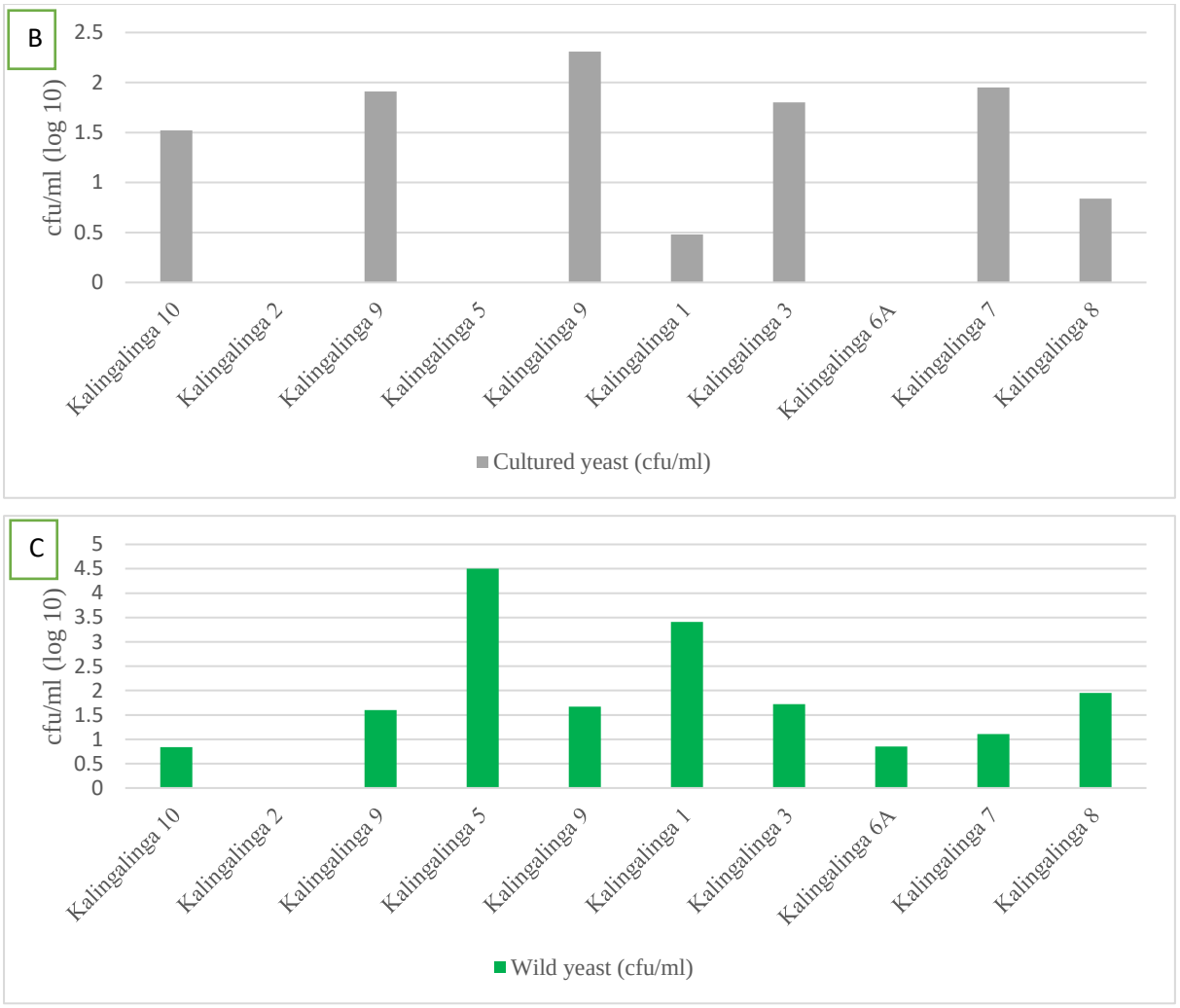


Figure 4.19: Baseline microbial results: A. the LAB count, B. cultured yeast count, and C. wild yeast counts in cfu/ml (log 10) of the *kachasu* mash.

4.3.2 Survey

Out of the 10 samples collected, seven (7) were detected for LAB, eight (8) had cultured yeast and all samples had wild yeasts. The results are shown in Figure 4.20.

N02, N04 and K 11 had no growth detected for LAB. Two (2) of these three (3) producers use wet maize malt (N02 and N04) and the third one uses dry malt. K06 and K11 had no growth detected for cultured yeast. N03 had the highest count of LAB, cultured yeast and wild yeast and the second highest alcohol content (61.40%) for the number 1 collection.

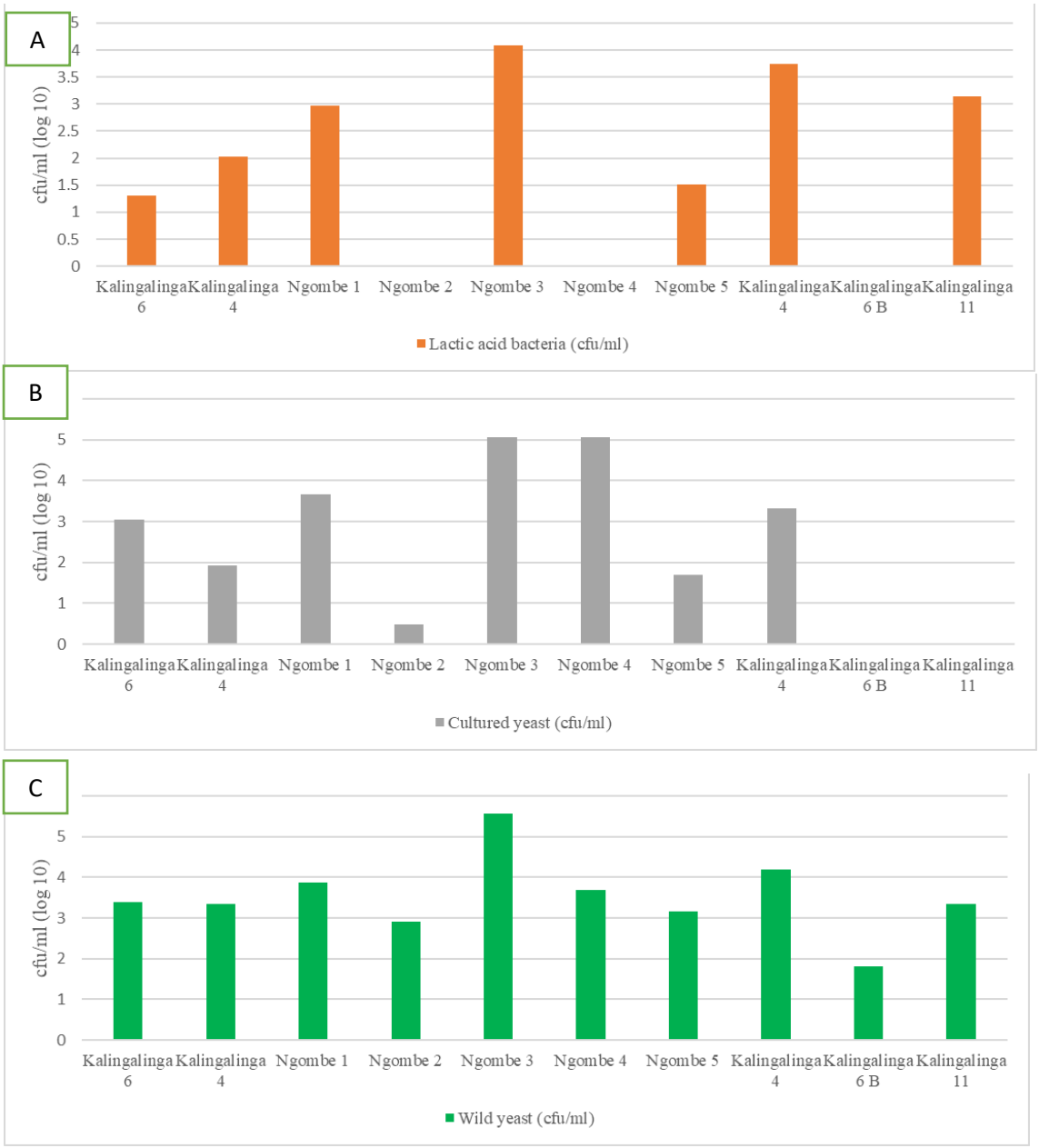


Figure 4.20: Microbial results for mash from Ng'ombe and Kalingalinga: A. LAB count. B. Cultured yeast count and C. Wild yeast count.

5.0 CHAPTER 5: DISCUSSION

The study identified the raw materials used to manufacture *kachasu* as table sugar, maize malt, water, and a starter culture from the previous batch. Sugar is the main carbohydrate source. This is procured from the local supermarkets and wholesalers. The quality of the table sugar is dependent on the major producers who include Zambia Sugar and Mansa sugar. The sugar is kept in its original packaging hence its integrity is not compromised. The sugar (sucrose) together with water, are fermented to ethanol and carbon dioxide in the absence of oxygen by the enzymes found in yeasts (Pepin et al, 2015). The water also acts as a medium for the sugar to dissolve and for extraction of sugars, bacteria, and yeasts from the maize malt which are essential for fermentation.

The study looked at the processing methods used to manufacture *kachasu* and found seven (7) distinct methods (Figure 4.3.). *Kachasu* processing starts with the production of maize malt. Maize is procured from the local market. The malting process relies on traditional knowledge, weather conditions and the quality of the maize. The ratio of water to maize for steeping is based on just enough to cover the maize grains in a plastic bucket. Steeping is done to initiate germination through absorption of moisture by the grain. Steeping is terminated when the grains are impregnated by water and a visual test is done. Some processors (K10) use old blankets as germination boxes as shown in Figure 4.1. The blanket keeps the maize grains moist, and water is regularly sprinkled on top to replenish the moisture. To allow for even germination, there is need for turning of the grains (Ndife et al, 2019). However, there was no evidence of turning of the grain at K10. The growth of the acrospire is measured visually. An acrospire is the first shoot developing from the plumule of a germinating grain seed (Felšöciová et al., 2020).The acrospire is allowed to grow excessively (Figure 4.1A) implying that very few sugars will be left as they would have been consumed by the shoot for it to grow. The storage conditions of the malt were not conducive for its integrity. The malt in Figure 4.2b had molds on it indicating that it was stored wet in a warm area with poor circulation of air. Molds result in the production of off flavors like mercaptans (Ndife et al, 2019).

The manufacture of *kachasu* can be modified by use of barley malt instead of maize malt since barley malt has a higher diastatic power. Diastatic power is the level of enzymes that are responsible for the conversion of starch to simple sugars in brewing. Also, barley has a shorter germination period compared to maize. This would have an impact on the flavor profile as barley malt has a different flavor from maize malt (Ohimain, 2016). This would result in a shorter malting period that would increase the production capacity of the *kachasu* processors.

Some processors (N01, N02, N03, N04, N05, K03. K04, K 06, K10) dry their malt before use as illustrated in Figure 4.1B. The figure shows that the drying area is not protected from animals and pests as a puppy is sleeping peacefully next to the malt after playing in the malt. Good manufacturing practices (GMPs) dictate that all food contact surfaces must be always in a sanitary condition (Gould et al, 1994). The puppies may contaminate the maize malt with pathogens like *Staphylococcus intermedius, Salmonella, Leptospira interrogans* and *Campylobacter*. Ingestion of s*almonella* results in several infectious diseases such as gastroenteritis, enteric fever, bacteremia and osteomyelitis. Gastroenteritis diseases are the most common clinical presentations of *Salmonella* in human and dogs though, most infected animals or humans are asymptomatic and may shed the pathogen through feces for a period of 6 weeks and transmit the pathogen to other animals or individuals(Ghasemzadeh et al, 2015). *Campylobacter* causes fever, diarrhea and vomiting in humans. *L. interrogans* causes leptospirosis which may present as fever, non-productive cough, vomiting, nausea, diarrhea, and muscular pain (Ghasemzadeh et al, 2015).

Control of these microorganisms may be at the distillation stage of the manufacturing process due to boiling of the mash to enable evaporation of the alcohol. The efficacy of the control needs to be established through further studies as the temperature is not measured during distillation. As a result, the effectiveness of the heating remains unknown. In addition to that, since tasting of the mash is done from day 1 of mixing, there is a possibility of transmission of the pathogens from the puppy to the processor through the maize malt from urine and fecal matter of the animal.

Some processors (K01, K05, K07, K11) omit the kilning step and use the germinated grains while the rest, kiln it by drying it in the sun to reduce its moisture content. Drying also halts the biological processes that will be taking place within the grain. Kilning by addition of heat would have resulted in a bit of caramelization that will impact on the flavor of the distilled *kachasu* (Ohimain, 2016). The maize malt is pounded to reduce its particle size. This enables effective extraction of sugars and enzymes from the maize malt as the surface area for contact with water is increased. Maize malt is a source of Lactic acid bacteria (LAB) and the number of microorganisms found depends on whether wet or dry malt is used (Udota, 2007).

Eight (8) processors utilize the backslopping technique, which is also utilized in the production of sauerkraut, sour dough bread (Nyanga et al., 2008a), and *kefir* (Kim et al., 2018). The sour mash utilized to produce *kachasu* may have a higher concentration of microorganisms compared to the cooked porridge which has been subjected to heat treatment during distillation. Some of these

microorganisms include yeasts and LAB that are essential for the fermentation process (Kim et al, 2018).

Fermentation time for the mash is influenced by the optimal fermentation conditions for yeast and LAB. The optimal fermentation temperature of traditional brewing yeast is 28–33°C, while optimal fermentation temperature for LAB is 30-40°C (Melikoglu et al, 2016). Since fermentation is carried out indoors, it must be very hot outside for the temperature to be ideal. Brewing yeast multiplication is very slow under low temperatures (Melikoglu et al., 2016) hence the longer fermentation time in the cold season. During fermentation, the processors periodically taste the mash for sweetness. Tasting for sweetness is a practice that is quick and requires the skill of the brewer to make the correct decision. In the production of *kanyanga* in Rwanda, fermentation is carried out for 4 to 7 days while the container is sealed. The test for the mash's readiness for distillation wasn't defined (Lyumugabe & Songa, 2019). Tasting is not an option since the fermentation vessels are sealed and a small hole opened when it is time to distill.

The *kachasu* manufacturing process in the two compounds under this study were found to have some similar process steps with *kachasu* from maize in Zimbabwe (Gadaga et al., 1999) as well as that of *kanyanga* from Burundi and *kanyanga* from Rwanda (Lyumugabe & Songa, 2019). The processes are all characterized by malting of a cereal grain, fermentation and distillation. The equipment used is also the rudimentary type and the processes rely on spontaneous fermentation, which lacks effective controls. It is also carried out under poor hygienic and unsterile environments (Ohimain, 2016; Nyanga et al., 2008b).

Use of secondhand PET bottles for the packaging of *kachasu* is a violation of the Potable Spirits Act of Zambia. The Act stipulates that a distilled liquor should be packaged in a glass bottle or new food grade materials that do not affect the product quality and safety ('SI-18-of-2020_-Portable-Spirits'). Cleaning of the bottles is done under unhygienic conditions and the effectiveness of the cleaning needs to be studied to understand its impact on the safety of *kachasu*. The highest alcohol content from the baseline study, of 71.12% is sufficient to disinfect pathogens. However, this is not the concentration at which *kachasu* is sold. In addition, this does not eliminate chemical impurities that could have penetrated or reacted with the plastic after beverage consumption. These chemicals would then be released into the *kachasu.* There were flies around the homestead and the water used for washing the bottles, was visually dirty. Hence there is a possibility of contamination of the *kachasu* by cleaning detergent and dirty water. Guidelines for cleaning of packaging material dictate

that bottles should be rinsed with potable water and sterilized prior to packaging of food (Gould et al, 1994).

The condition of the equipment used to manufacture *kachasu* was unsanitary across all the processors who took part in this study, from the malting blankets (Figure 3.1) to the fermentation drums (Figure 4.6). Some of the blue plastic drums used for fermentation had turned khaki in color. There was no evidence of cleaning of these drums from one batch to the next. This violates the *Potable Spirits* statute of Zambia which demands that any distilled liquor should be produced under hygienic conditions (SI-18-of-2020_-Portable-Spirits).

The type of metal that the condensation pipes were made from could not be identified. However, the metal drums were used oil drums sold at the market. The used motor oil bottles that are used to collect the distillate can possibly contaminate the *kachasu* as shown by the migration study carried out on HDPE bottles with 50% ethanol (Zimmermann, 2023). The setup of the equipment does not follow any GMP guidelines. The equipment is set up in the same premises where the cooking of food for the family is also normally done. The plastic used to cover the drum during distillation is black polythene sheeting that is made from recycled material. This also poses the risk of contamination of the liquor by chemicals which were packaged in the material before recycling (Zimmermann, 2023).

Only four (4) distillate samples had an alcohol content greater than the stipulated maximum of 55% by the SI 18 of 2020 of the Laws of Zambia. This implies that the alcohol content has the potential to be regulated by the producers in Zambia. It would be important to train them in a simple method of measuring the alcohol, for example the use of a hydrometer. This would eliminate the sale of high alcohol content *kachasu* which is detrimental to human health.

Since *kachasu* production is carried out under environmental conditions and is a result of spontaneous fermentation, the fermentation could be driven by homofermentative and heterofermentative LAB (Moonga et al., 2022). This results in the production of lactic acid, ethanol, and carbon dioxide (Egan et al., 1981). There is variation in the pH of *kachasu* from producer to producer throughout the three distillation stages. However, there is a decrease in pH from grade number 1 to number 3 of kachasu for all the processors. This could be a result of the increase in the water content as the alcohol content also decreases. Ethanol on its own is neutral, so addition or increase in water content results in a decrease in pH due to the increase in H^+ ions from the water (Egan et al., 1981).

In *kachasu,* the dissolved solids that contribute to the brix may be sugars from the table sugar, sugars from the maize malt, and some starch from the maize malt. The average brix for the mixed samples was as expected (5.7°Brix) since it contains a mixture of all the three collections. The mixed sample from N05 had a brix of zero, which was unexpected as it was supposed to have some dissolved solids.

The highest alcohol content from the survey was found to be 65.15% and this was a number 1 sample from N01 which is lower than that reported in unpublished studies reported by Africa Press in 2022 where it was reported to be 70%. This is however, higher than the 20% reported by the Lusaka times in 2022 in an article. In Zimbabwe, Tangwena distillery produces *masau, nyii* and *mazhanje kachasu* with a 40% alcohol content. They also produce *zumbani* and *resurrection bush* kachasu which have 30% alcohol (Tangwena, 2024). This is different from the Tanzanian *konyagi* which has 35%. *Konyagi* is also a commercialized traditional distilled liquor like *kachasu* (Madenge, 2022).

The number 1 grade is the most expensive to purchase since it has the highest alcohol content followed by the number 2 grade. The number 3 grade has the same price as the mixed one. The number 1 and number 2 grades are not usually sold as is. They are used to make the final retail sample.

The sugar concentration was plotted against the corresponding alcohol content for the number 1 distillate as shown in Figure 4.13. There was intermediate positive correlation meaning that an increase in sugar concentration results in an increase in alcohol content to a certain point where other factors will now determine the alcohol production. Alcohol production will also depend on the temperature of the mash as yeasts work more efficiently around 20 to 33°C and LAB 30 to 45°C (Egan et al., 1981). Yeast (*Saccharomyces cerevisiae)* and some species of LAB can withstand 160ml/l of ethanol hence their activity is not affected by increase in alcohol (Nyanga et al., 2007).

Production of traditional distilled liquors has been associated with methanol poisoning (Ohimain, 2016). The qualitative iodoform test carried out on all the distillate samples, tested positive for methanol in four samples N03(number 3), N05 (mixed), K04 (number 3), and K06 (number 3) out of 40 samples. These samples are also the ones that had the lowest alcohol content, 1.53%, 4.03%, 4.53%, 7.70%. The samples that tested negative for methanol may also contain aldehydes, methyl ketones and other secondary alcohols that contain the methyl group in the alpha position as they react in the same manner (Clark, 2015). Aldehydes and methyl ketones are prone to oxidation leading to a change in the flavor profile of the liquor. They are also mutagenic and carcinogenic

which makes them harmful to human health if consumed in large amounts. These compounds have stipulated limits as set by Statutory Instrument 18 of 2020 in Zambia hence there is need for further analysis to determine the compounds that are present and their quantities. This will enable the determination of the safety of *kachasu* with respect to their concentration. Methanol contamination has also been found in both low and high alcohol beverages as shown in Table 2.2 implying that this problem is not only unique to Zambia. Zambia can then take lessons from Kenya where traditionally distilled liquor is now regulated in order to try and eliminate contamination (Mkuu et al., 2019b). The method used to detect methanol in this study was a qualitative method that does not give insight into the quantities present. There is a need to further analyse the distillate using other quantitative methods like gas chromatography.

The distillate samples were analysed for their iron, cadmium and copper concentrations. The iron concentration range for number 1 (first grade) was 0.021 to 0.158µg/ml which is lower than the maximum permissible limit of 8mg/L('SI-18-of-2020_-Portable-Spirits'). The copper levels detected were also within the limits (maximum of 2mg/l) according to S.I 18 of 2020 of the laws of Zambia. This implies that the copper and iron levels found in the *kachasu* produced in Kalingalinga and Ng'ombe are safe for human consumption. The studies carried out on *kachasu* produced in Zimbabwe did not focus on the mineral composition. Most studies on traditional distilled liquor have been focusing on the contaminants like methanol poisoning (Ohimain, 2016) and the microorganisms involved in the fermentation stage (Mkuu et al., 2019a,Nyanga et al., 2008b).

Microbiological analysis yielded some interesting results. Of the 20 mash samples collected, 9 samples had no growth when cultured on MRS agar for lactic acid bacteria. These samples are from all the seven (7) processing methods that were identified. K06 had three samples, the starter and two mash samples from day 3 and day 7 of fermentation. The day 3 mash sample had 2.0×10^{1} cfu/ml indicating that LAB may be involved in the fermentation process at K06. The absence in the day 7 mash sample of K06 and starter culture (of K06) is not due to the ethanol concentration as LAB are tolerant to alcohol, even 25% for as long as it is reintroduced to an environment rich in nutrients like in backslopping (Pittet et al., 2011).

The mash samples were also cultured in wort agar which enables growth of cultured yeast of the *Saccharomyces cerevisiae* (Hutzler et al., 2015). Six samples had zero growth on their plates. N03 *matokoso* had the highest number of cfu/ml with 1.13×10^{5}. This sample was also rich in LAB, and wild yeasts. The presence of cultured yeast in 16 of the collected mash samples is of significance since all the processors denied using commercial yeast for fermentation though a few hinted that

others use this yeast. This implies that the processors use commercially produced cultured yeast but do not want to be associated with its use.

Since the manufacture of *kachasu* relies on spontaneous fermentation, 19 of the 20 samples had growth of wild yeasts which were cultured on lysine agar. Lysine promotes the growth of wild yeasts of the *non-saccharomyces* strains(Lin, 1975). However, K02 had no colony forming units. It is the only sample that had no growth. Unfortunately, this baseline processor was no longer available when the processing method questionnaire was administered. This could have provided some insight as to why there was no growth of wild yeasts.

This study was able to pave the way for further research into the production and physico-chemical composition of *kachasu*. A range of alcohol compositions was established, and several production methods identified. This information can be used as a base for the possibility of commercialization. However, due to the scarcity of resources, some of the methods used for analysis were not the best for example methanol detection and use of a pycnometer for alcohol. Other analysis methods like HPLC gives more accurate results. The sample size was also small because some processors were not keen to share about their illegal business for fear of prosecution.

CHAPTER 6: CONCLUSION

The *kachasu* manufactured in Kalingalinga and Ng'ombe townships was found to be made from maize malt, table sugar, water and sometimes backslopping was carried out to include a starter culture of either the fermented mash or porridge leftover after distillation. Seven (7) processing methods were identified in this study with main areas of variation being use of wet or dry maize malt, backslopping and sieving of the sour mash prior to distillation. The study also revealed that kachasu had 3 distinct grades which were number 1, number 2, number 3 and mixed.

Only two (2) samples from the baseline study and three (3) samples from the survey were found to have an alcohol content above the 55% stipulated by S.I. 18 of 2020 out a total of 40. The number 1 grade had the highest alcohol content followed by number 2. Four (4) distillate samples were found to be contaminated with methanol out of 40. However, there is need for further analysis as the method was qualitative and could not quantify the amount present. The iron, cadmium and copper concentrations for all the distillate samples were found to be within the regulation guidelines. LAB, cultured, and wild yeast were found in the mash before distillation. *Kachasu* is being produced and sold though it is an illegal substance.

6.1 Recommendations

This study identified areas or gaps for further research with regards to the chemical composition of the distillate and the microbial composition of the sour mash in the production of *kachasu.* As a result, the following actions are recommended.

- The study found methanol contamination in *kachasu*. However, its amount was not quantified. There is a need to carry out further analysis to quantify the methanol and other parameters like the methanol concentration, aldehydes and isopropanol since the test carried out also gives the same result for the mentioned compounds.
- Various organic substances have been identified in other distilled liquor as shown in Figure 2.3, hence there is need to further analyse the *kachasu* distillate possibly by Gas or Liquid Chromatography to determine which compounds make up its composition.
- Most distillated samples that were analyzed did not have any methanol contamination hence there is a potential to legalize *kachasu* and have its production and sale regulated by the S.I 18 of 2020- Potable Spirits Act of Zambia. Producers may sell to a distillery for further purification or can form a cooperative that then purifies and properly packages the *kachasu* for sale.

- LAB, cultured yeast, and wild yeast were found in the mash. There is a need to further identify the types and optimize the process of manufacturing *kachasu* to eliminate variability of the product.
- There is a need to carry out microbial analysis to determine the evolution of microbes during fermentation from day 1 to day 7.

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Appendix I: Questionnaire of Selection of Informants

Dear Respondent, I am Shungu Rudo Takayindisa, a student at The University of Zambia. I am carrying out a survey aimed at collecting indigenous knowledge on different ways of producing kachasu and the factors that influence its quality with a view of improving and optimizing its production at different scales for local communities. This study is a research project under the School of Agricultural Sciences. Any information shared will be used in confidence. Your name and location will not be published. I thank you for your participation.

Questionnaire No. ……. Date:……..

No.	Question	Response
1.	How old are you?	
2.	In which suburb do you stay?	
3.	What do you do for a living?	
4.	Do you know who consumes any alcoholic beverage?	
5.	Do you know kachasu?	
6.	Are there any kachasu manufacturers / processors that you know? If yes, how many?	
7.	Would you like to assist the researcher in identifying and introducing the researcher to the manufacturer(s) of kachasu and help in assuring them that the data we are collecting is just for research purposes?	

Appendix 2: Questionnaire for Processors on the Different Methods of Processing Kachasu (English version).

Dear Respondent, I am Shungu Rudo Takayindisa, a student at The University of Zambia. I am carrying out a survey aimed at collecting indigenous knowledge on different ways of producing kachasu and the factors that influence its quality with a view of improving and optimizing its production at different scales for local communities. This study is a research project under the Faculty of Agricultural Sciences. Any information shared will be used in confidence. Your name and location will not be published. I thank you for your participation.

Questionnaire No. ……. Date:……..

No.	Question	Response
1	**Demographics and Socioeconomics**	
1.1	Time	
1.2	Location	
1.3.	Main language used by respondent	*1.* Lozi *2.* Tonga *3.* Nyanja *4.* Bemba *5.* Other(specify)……..
1.4	Gender	*1.* Male *2.* Female
1.5	Do you manufacture and sell *kachasu?*	1. Manufacture and sell wholesale. 2. Manufacture and sell wholesale and retail. 3. Manufacture and sell retail.
1.6	How many people at this premise are involved in the manufacture of *kachasu?*	
1.7	Ages of the people involved	*1.* ≤18 *2.* 2. 19-29 *3.* 30-39 *4.* 40-49 *5.* 50-59 *6.* 60+
1.8	Level of Education	*1.* 1. No education *2.* Primary (Grade 7) *3.* Secondary (O-Level or A-Level) *4.* Vocational training *5.* Tertiary (diploma or degree) *6.* Other (specify).....
1.9	Who is the head of the household	*1.* Father *2.* Mother *3.* Child *4.* Grandmother *5.* Grandfather *6.* Aunt *7.* Uncle

		8. 8. Other (specify)...
1.10	Size of the household	*1.* 0-6 *2.* 6-10 3. >10 *4.* Other (specify)
2.	**Raw Materials, Recipe and Equipment**	
2.1.	What raw materials do you use to produce your kachasu? (Multiple response)	*1.* Maize *2.* Maize malt *3.* Pearl millet *4.* Finger millet *5.* Sugar 6. Yeast *7.* Water *8.* Sorghum *9.* Other(specify)…….
2.2.	What's your recipe	1. 2. 3. 4. 5. 6. 7. 8. 9.
2.3	What containers/equipment do you use for kachasu manufacture?	
2.4	How do you acquire the raw materials used in the production of kachasu?	
2.5	How do you store the raw materials?	
2.6	Where do you ferment the mash?	*1.* In the sun *2.* Under the shade *3.* In doors
2.7	How long do you ferment the mash?	*1.* 1 day *2.* 1-2 days *3.* 2-3 days *4.* 3-4 days *5.* 4-5 days 6. 5-6 days *7.* 6-7days *8.* Other (specify)……..

2.8	How do you know that the mash is ready for distillation?	
2.9	What's your water source for cooling?	
2.10	What is your water source for cooking	
3.0	**Kachasu Variants for Consumer market**	
3.1	How do you prepare *kachasu* for sale?	
3.2	If dilution is done, what are the ratios and how to you determine them.	
3.3	How many liters do you produce or sell per brew	
3.4	For how long have you been brewing kachasu?	
3.5	Would you mind if pictures of your processing equipment and fermenting vessels are taken?	1. Yes 2. No
3.6	Observations (to be written soon after departing the premises)	

Appendix 3: Manufacturer's questionnaire in Nyanja

Wokondedwa Woyankha, ndine Shungu Rudo Takayindisa, wophunzira pa The University of Zambia. Ndikuchita kafukufuku wofuna kusonkhanitsa chidziwitso cha anthu am'deralo pa njira zosiyanasiyana zopangira kachasu ndi zinthu zomwe zimakhudza khalidwe lake ndi cholinga chowongolera ndikuwongolera kupanga kwake pamiyeso yosiyanasiyana kwa anthu am'deralo. Kafukufukuyu ndi ntchito yofufuza pansi pa School of Agricultural Sciences. Chidziwitso chilichonse chogawidwa chidzagwiritsidwa ntchito mwachinsinsi. Dzina lanu ndi malo anu sizidzafalitsidwa. Ndikukuthokozani chifukwa chotenga nawo mbali.

Mafunso Ayi:…… Tsiku:……

Ayi	Funso	Kuyankha
1	**Chiwerengero cha anthu ndi Socioeconomics**	
1.1	Nthawi	
1.2	Malo	
1.3.	Chilankhulo chachikulu chogwiritsidwa ntchito ndi wofunsidwa	1.Lozi 2.Tonga 1. Nyanja 2. Bemba 3. Zina(Tchulani)……..
1.4	Kugonana	1.Mwamuna 2.Wamukazi
1.5	Kodi mumapanga ndi kugulitsa kachasu?	4. Kupanga ndi kugulitsa yogulitsa. 5. Kupanga ndi kugulitsa yogulitsa ndi ritelo. 6. Kupanga ndi kugulitsa malo ogulitsa.
1.6	Kodi ndi anthu angati pa mfundo imeneyi amene amagwira nawo ntchito yopanga kachasu?	
1.7	Zaka za anthu okhudzidwa	1.≤18 2. 19-29 3. 30-39 4. 40-49 5. 50-59 6. 60+
1.8	Mlingo wa Maphunziro	1. Palibe maphunziro 2.Primary (Grade 7) 3.Secondary (O-Level kapena A-Level) 4. Maphunziro a ntchito 5.Tertiary (dipuloma kapena digiri) 6.Zina (tchulani).....
1.9	Kodi mutu wa banja ndi ndani	1.Atate 2.Amayi 3.Mwana 4.Agogo(mukazi) 5.Agogo(mwamuna) 6.Azakhali 7.Amalume 8.Zina (tchulani) .....

1.10	Kukula kwa banja	1. 0-6 2. 6-10 3.>10 4. Zina (tchulani)
2.	**Zipangizo Zopangira, Chinsinsi ndi Zida**	
2.1.	Kodi mumagwiritsa ntchito zipangizo ziti popanga kachasu yanu? (Kuyankha kwambiri)	1. Chimanga 2. Chimanga chimera 3. Pearl mapira 4. Chala mapira 5. Shuga 6. Yisiti 7. Madzi 8. Manyuchi 9. Zina(tchulani).....
2.2.	Chinsinsi chanu ndi chiyani?	1. 2. 3. 4. 5. 6. 7. 8. 9.
2.3	Mumagwirits ntchito zotengera kapena zida ziti popanga kachasu?	
2.4	Mumapeza bwanji zopangira zomwe zimagwiritsidwa ntchito popanga kachasu?	
2.5	Mumasunga bwanji zopangira?	
2.6	Mumayatsa kuti phala?	1. Padzuwa 2. Pansi pa mthunzi 3. M'nyumba
2.7	Mumayatsa mpaka liti?	1. Wina tsiku 2. 1-2 masiku 3. 2-3 masiku 4. 3-4 masiku. 5. 4-5 masiku 6. 5-6 masiku 7. 6-7masiku 8. Zina(tchulani)……..
2.8	Mumadziwa bwanji kuti phala lakonzeka kuti distillation?	

2.9	Gwero lako la madzi oziziritsa ndi liti?	
2.10	Madzi anu ophikira ndichiyani?	
3.0	**Kachasu Zosiyanasiyana kwa ogula msika.**	
3.1	Mumakonzera bwanji kachasu zogulitsa?	
3.2	Ngati dilution yachitika, maratios ndichiyani ndipo mumawadziwa bwanji?.	
3.3	Mumagulitsa kapena kugulitsa malita angati pa mowa uliwonse?	
3.4	Mwakhala mukupanga kachasu kwanthawi yayitali bwanji?	
3.5	Mungadabwe ngati zithunzi zazida zanu zogwirira ntchito ndi zotengera zofufumitsa zijambu lidwa?	3. Inde. 4. Ayi.
3.6	Zowonera (kuti alembedwe atangochoka pamalopo)	

Appendix 4: Ethical clearance

TROPICAL DISEASES **RESEARCH CENTRE**

Tel/Fax +260212 615444
P O Box 71769
tdrc-ethics@tdrc.org.zm
NDOLA, ZAMBIA

TDRC RESEARCH ETHICS COMMITTEE
IRB REGISTRATION NUMBER : 00002911
FWA NUMBER : 00003729

TRC/C4/08/2023

29th August 2023

Takayindisa Rudo Shungu
University of Zambia
Dept of Food Science and Nutrition
LUSAKA

Dear Rudo,

RE: ETHICAL APPROVAL OF STUDY PROTOCOL

Reference is made to the protocol entitled **"Production Process, Phyico-Chemical and Microbiological Characterization of a Traditionally Distilled Liquor, Kachasu in Lusaka. TDREC/103/02/23**

On behalf of the Chairman of the TDRC Research Ethics Committee (REC), I am pleased to inform you that your protocol was reviewed and granted ethical approval based on conditions below.

You are advised provide a comprehensive information sheet and consent form which should be translated into a local language to cater for participants that may not be conversant with the English language. Further, ensure that page numbers and a corrected table of contents are included in the document.

Should there be any protocol modifications, amendments or violations, you are required to notify the REC and submit protocol amendments for approval.

You are now required to submit your protocol to the National Health Research Authority for final approval following the link**: https://www.nhra.org.zm.** A final report to the study should be submitted to the REC Secretariat at the end of the study.

This approval is valid for the period, **29th August 2023** to **29th August 2024**

The Committee wishes you success in academic work and execution of the study.

Yours faithfully,
TROPICAL DISEASES RESEARCH CENTRE

Edna Mwale Simbayi
SECRETARY – TDRC Ethics Review Committee

Appendix 5: Standard curves and results

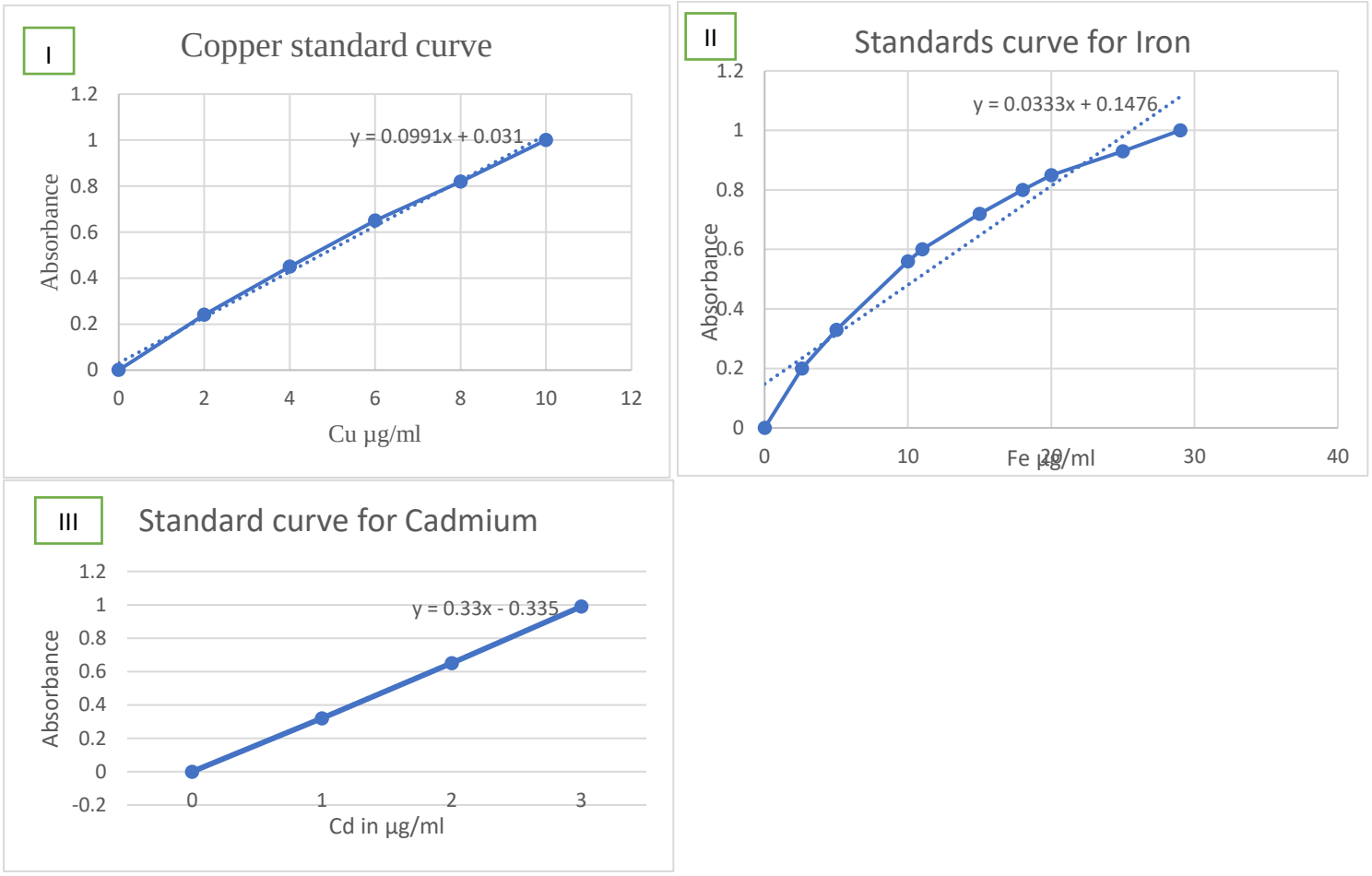

Figure a: Standard curves for mineral determination. I is for copper, II for iron and III for cadmium

Appendix 6: Detailed chemical and microbiological results.

Table A6.1: pH distribution for *kachasu* distillate collected during the survey.

Processor	Number 1	Number 2	Number 3	Mixed
Ng'ombe 1	3.61	3.42	3.11	3.45
Ng'ombe 2	3.65	3.4	3.28	3.35
Ng'ombe 3	3.91	3.5	3.27	3.23
Ng'ombe 5	3.45	3.33	3.13	3.11
Ng'ombe mean	**3.66 ±0.19**	**3.41 ±0.07**	**3.20 ±0.09**	**3.29 ±0.15**
Ng'ombe range	**3.45 – 3.91**	**3.33 -3.5**	**3.11 – 3.27**	**3.11 – 3.45**
Kalingalinga 4	4.13	3.45	3.24	3.4
Kalingalinga 6	3.68	3.54	3.33	3.33
Kalingalinga 11	4.1	4.21	3.69	3.28
Kalingalinga mean	**3.97 ±0.26**	**3.73 ±0.42**	**3.42 ±0.24**	**3.34 ±0.06**
Kalingalinga range	**3.68 -4.13**	**3.45 -4.21**	**3.24 – 3.69**	**3.28 -3.40**
Overall mean	**3.79 ± 0.26^{a,}**	**3.55 ± 0.30^{ab}**	**3.29 ± 0.19^{c}**	**3.31 ± 0.11 ^{bd}**
Overall range	**3.45 – 4.13**	**3.33 – 4.21**	**3.11 – 3.69**	**3.11 -3.45**

NB: Means with the same letters not significantly different according to LSD at 0.05 level.

Table A6.2: Brix (°) analysis for the distillate (*kachasu)* collected during the survey.

Processor	Number 1	Number 2	Number 3	Mixed
Ng'ombe 1	7.6	6	6.2	5.4
Ng'ombe 2	15.8	16.2	8.4	7.2
Ng'ombe 3	16.4	8.4	0.4	3.8
Ng'ombe 5	15	8	10	0
Ng'ombe mean	**13.7 ±4.1**	**9.7 ±4.5**	**6.3 ± 4.2**	**4.1 ±3.1**
Ng'ombe range	**7.6 – 16.4**	**6 – 16.2**	**0.4 -8.4**	**0 -7.2**
Kalingalinga 4	14.4	9.6	1	7.8
Kalingalinga 6	16.2	8.2	1.2	8
Kalingalinga 11	15.2	12.4	7.2	7.4
Kalingalinga mean	**15.2 ±0.90**	**10.1 ±2.1**	**3.1 ± 3.52**	**7.7 ±0.31**
Kalingalinga range	**14.4 -16.2**	**8.2 -12.4**	**1 – 7.2**	**7.4 – 8.0**
Overall mean	**14.37 ± 3.07[a]**	**9.8 ± 3.4[b]**	**4.9 ± 4.0[c]**	**5.66 ± 2.9[c]**
Overall range	**7.6 – 16.4**	**6.0 – 16.2**	**0.4 - 10**	**0 – 8.0**

NB: Means with the same letters not significantly different according to LSD at 0.05 level.

Table A6.3: Total acidity (%, m/v) by manufacturer for *kachasu* distillate.

Processor	Number 1	Number 2	Number 3	Mixed
Ng'ombe 1	0.05	0.12	0.17	0.09
Ng'ombe 2	0.06	0.13	0.19	0.11
Ng'ombe 3	0.05	0.08	0.11	0.10
Ng'ombe 5	0.10	0.14	0.12	0.27
Ng'ombe mean	**0.065±0.02**	**0.12 ±0.03**	**0.15 ±0.04**	**0.14 ±0.09**
Ng'ombe range	**0.05 -0.10**	**0.08 – 0.14**	**0.11 – 0.19**	**0.09 – 0.27**
Kalingalinga 4	0.06	0.12	0.17	0.12
Kalingalinga 6	0.10	0.06	0.10	0.07
Kalingalinga 11	0.09	0.02	0.02	0.14
Kalingalinga mean	**0.08 ±0.02**	**0.07 ±0.05**	**0.10 ±0.08**	**0.11 ±0.04**
Kalingalinga range	**0.06 – 0.10**	**0.02 – 0.12**	**0.02 – 0.17**	**0.07 -0.14**
Mean	**0. 07 ± 0.02[a]**	**0.09 ± 0.04[a]**	**0.13 ± 0.06[a]**	**0.13 ± 0.06[a]**
Range	**0.05 – 0.10**	**0.02 – 0.14**	**0.02 – 0.19**	**0.02 – 0.27**

NB: Means with the same letters not significantly different according to LSD at 0.05 level.

Table A6.4: Alcohol content (%v/v) of the *kachasu* distillates according to grades.

Processor	Number 1	Number 2	Number 3	Mixed
Ng'ombe 1	65.15	18.49	18.94	33.99
Ng'ombe 2	54.59	54.85	24.86	23.65
Ng'ombe 3	61.40	37.29	1.53	10.63
Ng'ombe 5	47.96	37.30	18.55	7.70
Ng'ombe mean	**57.28 ± 7.59**	**36.98 ± 14.85**	**15.97 ±10.05**	**18.99 ± 12.17**
Ng'ombe range	**47.96 – 65.15**	**18.49 – 54.85**	**1.53 – 24.86**	**7.70 – 33.99**
Kalingalinga 4	44.66	28.04	4.03	22.31
Kalingalinga 6	52.43	23.80	4.53	24.62
Kalingalinga 11	45.19	35.99	21.98	36.87
Kalingalinga mean	**47.43 ± 4.34**	**29.28 ±6.19**	**10.18 ±10.22**	**27.93 ±7.83**
Kalingalinga range	**44.66 – 52.43**	**23.80 – 35.99**	**4.03 – 21.98**	**22.31 – 36.87**
Mean	**53.05 ± 7.93^{a}**	**33.68 ±11.83^{bd}**	**13.49 ± 9.74^{c}**	**22.82 ±10.83 ^{cd}**
Range	**44.66 – 65.15**	**18.49 -54.85**	**1.53 – 24.86**	**7.70 – 36.87**

NB: Means with the same letters not significantly different according to LSD at 0.05 level.

Table A6.5: LAB, cultured yeast and wild yeast colony counts for the baseline study.

Sample	Days of fermentation	Lactic acid bacteria (cfu/ml)	Cultured yeast (cfu/ml)	Wild yeast (cfu/ml)
Kalingalinga 10	1	2.83×10^2	3.3×10^1	7
Kalingalinga 2	3	ND	ND	ND
Kalingalinga 9	3	6.3×10^1	8.3×10^1	4.0×10^1
Kalingalinga 5	4	ND	ND	3.15×10^4
Kalingalinga 9	4	5.13×10^2	2.03×10^2	4.7×10^1
Kalingalinga 1	7	9.7×10^1	3	2.55×10^3
Kalingalinga 3	**7**	ND	6.3×10^1	5.3×10^1
Kalingalinga 6A	**7**	ND	ND	7
Kalingalinga 7	7	ND	ND	1.3×10^1
Kalingalinga 8	7	3.47×10^2	7	9.0×10^2

NB: ND means non-detected.

Table A6.6: LAB, cultured yeast and wild yeast colony counts for survey.

Sample	Days of fermentation	Lactic acid bacteria (cfu/ml)	Cultured yeast (cfu/ml)	Wild yeast (cfu/ml)
Kalingalinga 6	3	2.0×10^1	1.093×10^3	2.46×10^3
Kalingalinga 4	4	1.07×10^2	8.4×10^2	2.25×10^3
Ng'ombe 1	7	9.47×10^2	4.553×10^3	7.35×10^3
Ng'ombe 2	7	ND	3	8.30×10^2
Ng'ombe 3	7	1.19×10^4	1.13×10^5	3.6×10^5
Ng'ombe 4	7	ND	1.713×10^3	4.99×10^3
Ng'ombe 5	7	3.3×10^1	4.9×10^2	1.417×10^3
Kalingalinga 4	7	5.543×10^3	2.067×10^3	1.575×10^4
Kalingalinga 6 B	7	ND	ND	6.7×10^1
Kalingalinga 11	7	1.407×10^3	ND	2.23×10^3

NB: ND means that no growth was detected.

Table A6.7: Iron content (μg/ml) for *kachasu* by distillation collection stage.

Processor	Number 1	Number 2	Number 3	Mixed
Ng'ombe 1	0.053	ND	ND	ND
Ng'ombe 2	0.021	ND	0.181	0.098
Ng'ombe 3	0.084	ND	0.052	ND
Ng'ombe 5	0.158	ND	ND	ND
Ng'ombe mean	**0.079 ±0.059**	**0±0**	**0.0582 ±0.0854**	**0.0245 ±0.049**
Ng'ombe range	**0.021 – 0.158**	**0**	**0 – 0.181**	**0 – 0.098**
Kalingalinga 4	0.031	ND	0	0
Kalingalinga 6	0.039	ND	0.115	0.021
Kalingalinga 11	0.117	ND	0	0.051
Kalingalinga mean	**0.062±0. 048**	**0**	**0.0383±0.066**	**0.024±0.026**
Kalingalinga range	**0.031 – 0.117**	**0**	**0 – 0.115**	**0 – 0.051**
Overall mean	**0.07 ±0.05^{a}**	**-^{bc}**	**0.05 ±0.07^{ab}**	**0.02 ±0.04^{c}**
Overall range	**0.02 – 0.158**	**-**	**ND – 0.181**	**ND – 0.098**

NB: ND means non-detected. Means with the same letters not significantly different according to LSD at 0.05 level.

Table A6.8: Copper content (µg/ml) for the distillate (*kachasu).*

Processor	Number 1	Number 2	Number 3	Mixed
Ng'ombe 1	0	0.011	0.011	0.01
Ng'ombe 2	0.011	0.004	ND	ND
Ng'ombe 3	ND	ND	ND	ND
Ng'ombe 5	ND	ND	ND	ND
Ng'ombe mean	**0.0027 ±0.005**	**0.00375±0.005**	**0.00275±0.006**	**0.0025±0.005**
Ng'ombe range	**0 – 0.011**	**0 – 0.011**	**0 – 0.011**	**0 – 0.01**
Kalingalinga 4	ND	ND	ND	ND
Kalingalinga 6	ND	ND	ND	ND
Kalingalinga 11	ND	ND	ND	ND
Kalingalinga mean	**0**	**0**	**0**	**0**
Kalingalinga range	**0**	**0**	**0**	**0**
Mean	**0.002 ±0.004[a]**	**0.002 ±0.004[a]**	**0.002 ±0.004[a]**	**0.001 ±0.004[a]**
Range	**ND – 0.01**	**ND – 0.01**	**ND -0.01**	**ND- 0.01**

NB: Means with the same letters not significantly different according to LSD at 0.05 level.

Appendix 7: Statistical analysis results

For this section, the following key applies:

- μ1 refers to the mean of number 1 samples.
- μ2 is the mean of number 2 samples.
- μ3 is the mean of number 3 samples.
- μ4 is the mean of the mixed samples.
- μN is the mean of Ng'ombe samples.
- μK is the mean of Kalingalinga samples.

I. pH

H_0 : μ1 = μ 2= μ3 = μ4

H_1 : μ1 ǂ μ2 ǂ μ3 ǂ μ4

Table A7.1: ANOVA for pH number 1, number 2 and number 3 and mixed

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.160	3	0.387	7.480	0.00106	3.009
Within Groups	1.241	24	0.052			
Total	2.403	29				

F (7.480) is greater than F critical (3.009) therefore we reject the null hypothesis and conclude that the means for the 4 groups are not equal.

An area analysis was carried out to determine if there was a difference between Ng'ombe and Kalingalinga.

$H_0 : \mu N = \mu K$

$H_1 : \mu N \neq \mu K$

Table A7.2: ANOVA for pH Ng'ombe versus pH Kalingalinga

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.103513	1	0.103513	1.659254	0.245147	5.987378
Within Groups	0.37431	6	0.062385			

F (1.659254) is less than F critical (5.987378), therefore there is no significant difference between the pH of Ng'ombe and that of Kalingalinga.

II. Brix

$H_0 : \mu_1 = \mu_2 = \mu_3 = \mu_4$

$H_1 : \mu 1 \neq \mu 2 \neq \mu 3 \neq \mu 4$

Table A7.3: Brix ANOVA for number 1, number 2, number 3 and mixed samples

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	374.941	3	124.981	10.812	0.0001098	3.00878
Within Groups	277.417	24	11.5591			
Total	652.358	27				

F (10.81) is greater than F critical (3.008) therefore we reject the null hypothesis and conclude that the means for the 4 groups are not equal.

An analysis was also done to determine if there was a difference between the brix of Ng'ombe and that of Kalingalinga. The ANOVA is shown in Table d.

$H_0 : \mu N = \mu K$

$H_1 : \mu N \neq \mu K$

Table A7.4: ANOVA for Ng'ombe vs Kalingalinga brix

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.78125	1	0.78125	0.036286	0.855209	5.987378
Within Groups	129.1836	6	21.5306			

F (0.036) is less than F critical (5.99) therefore there is no significant difference between the brix content of Ng'ombe and Kalingalinga samples.

III. Alcohol

$H_0 : \mu_1 = \mu_2 = \mu_3 = \mu_4$

$H_1 : \mu 1 \neq \mu 2 \neq \mu 3 \neq \mu 4$

Table A7.5 : Alcohol % ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6068.062	3	2022.687	19.496	0.000001.268	3.009
Within Groups	2490.012	24	103.751			
Total	8558.074	27				

F (19.496) is greater than F critical (3.009) therefore we reject the null hypothesis and conclude that the means for the 4 groups are not equal.

H_0 : μN = μK

H_1 : μN ǂ μK

Table A7.6: Alcohol content comparison between Ng'ombe and Kalingalinga

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	25.932	1	25.932	0.087214	0.777703	5.987378
Within Groups	1784.033	6	297.3389			
Total	1809.965	7				

F(0.087) is less than F critical (5.987) therefore we do not reject the null hypothesis and conclude that there is no significant difference between the alcohol content of samples from Ng'ombe and Kalingalinga.

IV. Total acidity as Lactic acid

$H_0 : \mu 1 = \mu 2 = \mu 3 = \mu 4$

$H_1 : \mu 1 \neq \mu 2 \neq \mu 3 \neq \mu 4$

Table A7.7: Total Acidity as lactic acid ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.015101	3	0.005034	1.952679	0.148084	3.008787
Within Groups	0.061867	24	0.002578			
Total	0.076967	27				

F (1.95) is less than F critical (3.01) therefore we do not reject the null hypothesis and conclude that there is no significant difference between the means of the four groups.

$H_0 : \mu N = \mu K$

$H_1 : \mu N \neq \mu K$

Table A7.8: Total acidity comparison between Ng'ombe and Kalingalinga

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.001677	1	0.001677	1.895021	0.217799	5.987378
Within Groups	0.00531	6	0.000885			
Total	0.006987	7				

F (1.895) is less than F critical (5.987) hence we do not reject the null hypothesis and conclude that there is no significant difference between the total acidity of samples from Ng'ombe and Kalingalinga.

V. Iron

$H_0 : \mu 1 = \mu 2 = \mu 3 = \mu 4$

$H_1 : \mu 1 \neq \mu 2 \neq \mu 3 \neq \mu 4$

Table A7.9: Iron concentration ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.020343	3	0.006781	2.94809873	0.053122	3.008787
Within Groups	0.055204	24	0.0023			
Total	0.075547	27				

F (2.95) is less than F critical (3.009) therefore we do not reject the null hypothesis and conclude that there is no significant difference between the means of the 4 groups.

$H_0 : \mu N = \mu K$

$H_1 : \mu N \neq \mu K$

Table A7.10 : Iron content comparison between Ng'ombe and Kalingalinga samples.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000172	1	0.000172	0.179715	0.686396	5.987378
Within Groups	0.005739	6	0.000956			
Total	0.005911	7				

F(0.179) was less than F critical (5.987) therefore we do not reject the null hypothesis and conclude that the is no significant difference between the iron content of Ng'ombe and Kalingalinga samples.

VI. Copper concentration.

$H_0 : \mu 1 = \mu 2 = \mu 3 = \mu 4$

$H_1 : \mu 1 \neq \mu 2 \neq \mu 3 \neq \mu 4$

Table A7.11: Copper concentration ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.00000211	3	0.000000702	0.04235463	0.9881	3.008787
Within Groups	0.000398	24	0.0000166			
Total	0.000405	29				

F (0.04) is smaller than F critical (3.009) therefore we do not reject the null hypothesis and conclude that there is no significant difference between the means of the four groups.

H_0 : μN = μK

H_1 : μN ǂ μK

Table A7.12: Copper concentration comparison for samples from Ng'ombe and Kalingalinga

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.73×10^{-05}	1	1.73×10^{-05}	112.322	4.15×10^{-05}	5.987378
Within Groups	9.22×10^{-07}	6	1.54×10^{-07}			
Total	1.82×10^{-05}	7				

F (112.322) is greater than F critical (5.987) therefore we reject the null hypothesis and conclude that there is a significant difference between the copper concentration of samples from Ng'ombe and Kalingalinga.

VII. LSD calculations

The following formula was used to calculate the LSD.

$LSD_{(A,B)} = t_{0.05/2,\ DFW} \times \sqrt{(MSW(1/n_a + 1/n_b))}$

Where LSD is

t = critical value from the t-distribution table

MSW = mean square within, obtained from the results of your ANOVA test

n = number of scores used to calculate the means.

Table A7.13: LSD calculations

	Brix		pH		Alcohol	
	Mean difference	LSD	Mean difference	LSD	Mean difference	LSD
Number 1 and Number 2	4.60*	0.332	0.24**	0.25	19.37*	11.24
Number 1and Number 3	9.51*	0.332	0.50*	0.25	39.57*	11.24
Number 1 and Mixed	8.03*	0.332	0.48*	0.25	30.23*	11.24
Number 2 and Number 3	4.91*	0.332	0.26*	0.25	20.19*	11.24
Number 2 and Mixed	3.43*	0.332	0.24**	0.25	10.85**	11.24
Number 3 and Mixed	1.49*	0.332	0.01**	0.25	13.49*	11.24

Note: * mean difference is greater than LSD so we reject the null hypothesis and conclude that the means are different. ** the mean difference is less than LSD, so we accept the null hypothesis that the means are the same.