RISK ASSESSMENT OF THE PRODUCTION OF THE ZAMBIAN FERMENTED MAIZE DRINKS MUNKOYO AND CHIBWANTU

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Abbreviations

Abbreviation	Definition
BLAST	Basic Local Alignment Search Too
BGA	Brilliant Green agar
BHIA	Brain Heart Infusion agar
Bm	Burnt munkoyo/Northern-type
BPA	Baird-Parker agar
Ci	Chibwantu
HPLC	High-Performance Liquid Chromatography
LB-broth	Luria-Bertani broth
MRS	De Man, Rogosa and Sharpe agar
Mu	Munkoyo/Central/Eastern-type
MYP	Mannitol Egg Yolk Polymyxin Agar
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose agar
SSA	Salmonella Shigella agar
TSI	Triple Sugar Iron
VRBGA	Violet Red Bile Glucose Agar

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Summary

A risk assessment was made of the fermented maize-based drinks munkoyo and chibwantu from Zambia. This assessment comprises three parts: the resilience against and presence of selected pathogens in the product , a review of possibly present toxic compounds in the product and the perception of consumers and producers on the safety of the product. The resilience of the microbial community in munkoyo against invasion of selected pathogens was tested over four days of fermentation at 25 °C and during storage in the finished product at 4 °C. The product did not show to be resilient; however the number of *S. aureus* and *B. cereus* reduced by 0.8 log CFU/ml and 2.9 log CFU/ml respectively over fermentation. 4 of 5 pathogens reduced between 0.5 and 2.11 log CFU/ml during the period of storage. When tested, samples that were taken in Zambia revealed that 15 out of 16 samples were contaminated with *S. aureus, B. cereus* or Enterobacteriaceae. All samples contaminated with *S. aureus* contained the minimal infective dose for enterotoxin production. For *B. cereus* only half of the samples contained the infective dose. However, for all samples a low pH was probably reached rapidly during fermentation, making it unlikely for the organisms to cause any adverse effects in form of toxin production.

The samples from Zambia were analyzed using HPLC on their ethanol, sugar and acid content. Ethanol could be found in all samples tested, with 5 out of 16 samples even passing the amount that is considered “non-alcoholic” (<0.5 % v/v) . Furthermore lactic acid was found in all samples. Acetic acid, formic acid and citric acid were found in some of the samples. Furthermore glucose, maltose, sucrose, mannitol and fructose were found in varying concentrations.

The majority of consumers do not associate any risks with munkoyo/chibwantu. Gaining trust is important when marketing the product by being transparent about the production and product. Bigger scale producers can gain trust by using transparent bottles, by allowing people to visit the production site and by keeping a social media page in which the process is shown and consumers can ask questions. The most reported symptoms of illness after consumption of the product are related to food poisoning and intoxication and comprise abdominal cramps, diarrhea and vomiting.

Several measures or actions are already taken by producers to ensure a safe production. However there is a lot of room for improvement that can already be implemented at the household scale of production. The most important measure is the necessity to cool down the maize gruel more rapidly. Furthermore manual contact with the product after cooking should be avoided at all costs. Water that is used for cleaning or producing should always be heat treated or purified in another way, especially if it will be added to the product after the heating and fermentation steps. A test should be developed to ensure that the right munkoyo root is used.

General introduction

Fermented foods in Africa

Throughout Africa many different fermented products exist. These products are often consumed on a daily basis and supply a significant amount of the energy and nutrients needed for a balanced diet (Odunfa, 1988). Fermenting food changes the flavor and texture, but it is not only interesting from an organoleptic perspective. Through fermentation, the nutritional value and digestibility of foodstuffs is increased. Furthermore the product becomes more stable against spoilage, even at ambient temperatures, through various mechanisms which are further explained in the next paragraph. Fermentation usually does not require a lot of expensive or complicated processing, which makes it a preservation technique that is suitable even in the most remote areas. In this thesis the focus will be on the Zambian fermented maize drinks munkoyo and chibwantu.

Munkoyo/chibwantu are maize-based drinks to which a special root, called the munkoyo root, is added. The drink is consumed as a source of energy or during festivities. As mentioned before, fermentation can increase the microbial stability of foodstuffs. In munkoyo/chibwantu, this happens through lowering of the pH and the consumption of potential substrates for growth of pathogens by the microorganisms performing the fermentation. The acids produced interfere with the maintenance of the pH gradient across the cytoplasmic membrane and inhibit active transport (Gram et al 2002 en Blandino et al 2003). Furthermore, other antibacterial compounds like ethanol, carbon dioxide, diacetyl and hydrogen peroxide are formed (Adams & Nicolaides, 1997). Bacteriocins are possibly produced and there are suspicions that the munkoyo root also contains antibacterial compounds (Mwale, 2014). Even though fermentation is supposed to make products safer, there are numerous reported cases of hospitalization after munkoyo consumption reported each year, which is further elaborated on in chapter 3.

The process

Munkoyo/chibwantu can be made in different ways. Three distinct methods exist: the central/eastern-type, referred to as munkoyo or mu in this thesis, the southern-type, referred to as chibwantu or chi, and the northern type, referred to as burnt munkoyo or bm (see figure 1). All processes start with maize milled to different degrees of coarseness. The water is heated, the maize added and the mixture is cooked for between 45 minutes and 3 hours. After cooling down the porridge, munkoyo roots are either added directly or added after being soaked in water. In case of soaking only the liquid is added to the porridge. The roots add flavor, amylases and microorganisms that subsequently ferment the maize porridge. The whole mixture is fermented overnight, usually in a calabash or plastic bucket. During the hot season the fermentation is finished the next morning. From that point on, it is either directly served or sold from the calabash, or it is taken to the market where it is sold in bottles that can be supplied by the sales(wo)man or in containers brought by the customers. Chibwantu differs from this process by using coarser maize grids. To make burnt munkoyo, the milled maize is deliberately burnt at the beginning of the processes.

Risk assessment

Even though munkoyo/chibwantu is usually consumed without causing any adverse reactions, there are cases of illness, hospitalization and even death (see chapter 3, introduction) that have been connected to the drink. This can be due to unhygienic handling or production, but also due to the

addition of the munkoyo roots after boiling of the porridge. They can add pathogenic bacteria to the porridge that will undergo no more subsequent heating steps. It is also suspected that if the wrong root is used, plant toxins are added to the product, which can cause various symptoms. These symptoms can vary from minor inconveniences like vomiting to more serious ones like affecting the nervous system. Little is known about these roots and their effect on the safety of the product: if by accident the root of another plant is taken, it can have adverse effects on the health of the consumer. Furthermore the temperature and humidity in Zambia can reach high levels, which increase the survival chance of pathogenic bacteria or growth of moulds on the raw materials (Roy et al 2007).

This thesis focuses on making a risk assessment on the production of munkoyo/chibwantu. This was done by identifying hazardous steps in the process. The severity of these hazards was assessed both experimentally and by reviewing literature. The outcome was laid down into a drawn and written recommendation for home producers to describe safe preparation of the product. Further recommendations were added for producers that want to upscale their production in terms of safe production, but also in terms of marketing. The recommendations were furthermore summarized in a HACCP-plan that could be used as a guideline for future production sites.

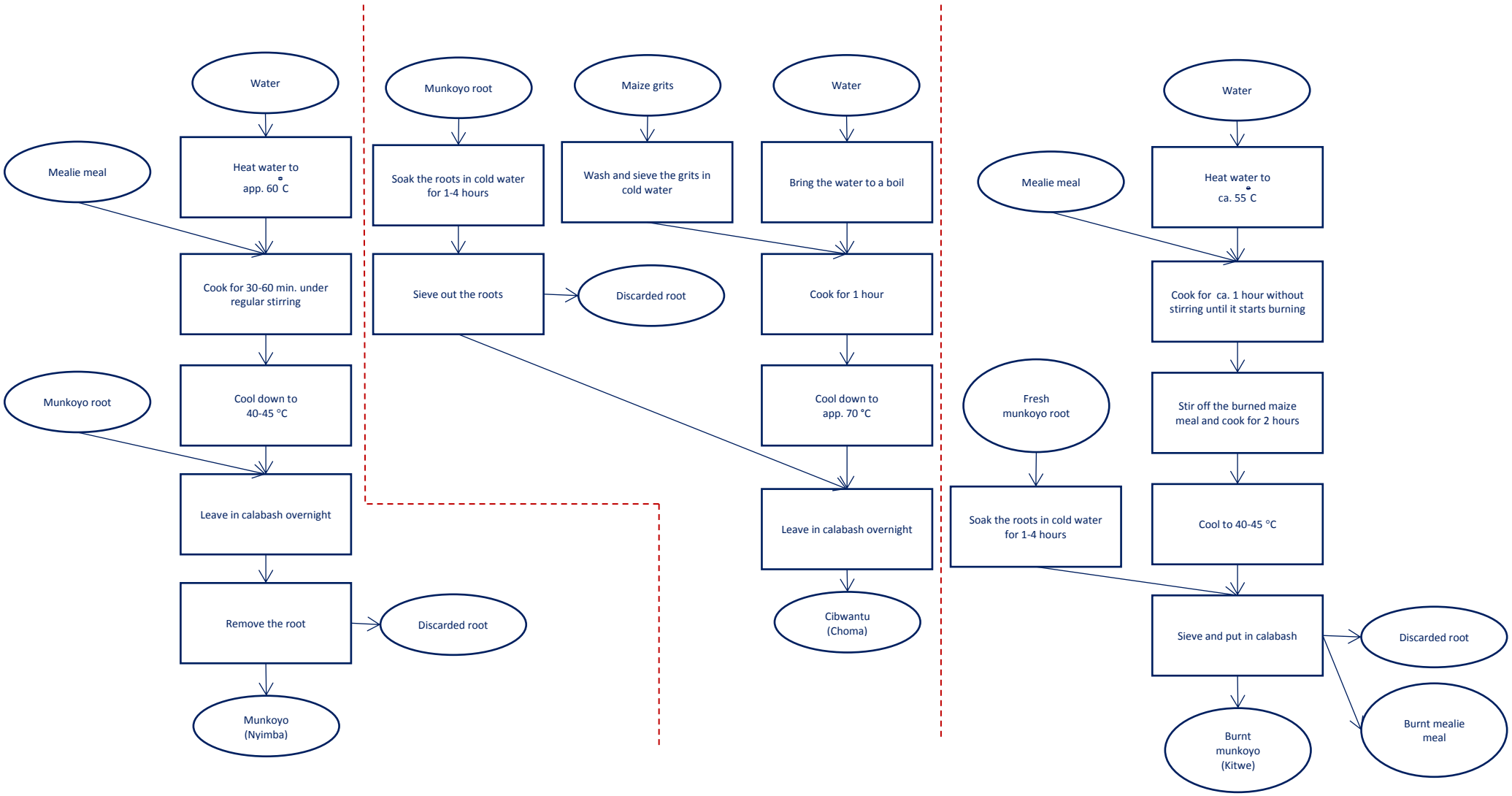


Figure 1 Production methods for munkoyo (central/eastern), chibwantu (southern) and burnt munkoyo (northern). Adapted figure from Sydney Phiri, obtained via personal communication on 13/9/2017.

Research questions and hypotheses

The aim of this research is to perform a risk assessment on the munkoyo production process. This was done by testing the resilience of the product against pathogen invasion and by identifying where in the process contamination of the product by pathogens could occur. Furthermore the product was screened for various compounds that can contribute to or reduce its safety. For the components that could not be tested on their presence due to time or budget restrictions a literature review was done. Last, both consumers and producers were interviewed to determine other possible risks and whether they perceive any risks related to the production of munkoyo.

The risk assessment was done by answering the following research questions. Each chapter represents a main question and is divided into subchapters by using sub-questions.

- **Chapter 1:** How resilient is munkoyo/chibwantu against the invasion of selected pathogens?
    - Do selected pathogens survive in munkoyo/chibwantu that is fermented at 25 ˚C for four days?
    - Do selected pathogens survive in fermented munkoyo/chibwantu during storage at 4 ˚C for four days?
    - What fraction of samples taken from Zambia is actually contaminated with pathogens and with which pathogens?
- **Chapter 2:** What other substances can be found in munkoyo/chibwantu and do these contribute to the safety of the product?
    - Is there ethanol in munkoyo/chibwantu, at what concentration and is this harmful to the health of the consumer?
    - Which acids can be found in munkoyo/chibwantu, at what concentration and do these contribute to the safety of the product?
    - Are there any sugars present in munkoyo/chibwantu, at what concentration and do these reduce or contribute to the safety of the product?
- **Chapter 3:** Do consumers and producers perceive any risks related to the production of munkoyo?

    Consumers
    - Is the product perceived as safe and what are the major perceived risks?
    - Do consumers ever experience symptoms of illness after drinking the product and what kind of symptoms are these?
    - What is important when marketing the product concerning the perceptions of risk?

    Producers
    - What are current risks in the production process and how can they be reduced?
    - Do producers perceive any risks in producing munkoyo/chibwantu and what do producers currently do to ensure a safe product?

Chapter 1: resilience against pathogens and presence of pathogens in munkoyo/chibwantu

Introduction

During boiling of the porridge all microorganisms present in the raw materials are eliminated. The microbes for fermentation therefore must come from the fermentation vessel, the munkoyo roots, the hands of the producer and the direct surroundings of the production process. Apart from the desired microbes, these sources can also introduce unwanted microbes into the product. For example *S. aureus*, which is found underneath the finger nails and in the noses of 30 percent of the world's population (Wertheim et al., 2005) is often introduced into food stuffs due to physical handling. Another source of pathogens can be water that is used during the process. Water can introduce all kinds of pathogens if contaminated, like *Shigella, Salmonella, V. cholerae.* Furthermore, the roots introduce a risk since they are taken from the soil and are actually proven to contain Enterobacteriaceae (Mwale, 2014), which indicates that the root can indeed introduce microbes that originate from the guts of animals (Ghafir et al., 2008). Besides introduction of pathogens after the boiling of the porridge, pathogens can even enter the product previous to boiling. Some pathogens have the potential to survive the boiling process through forming so-called endospores. These endospores are resistant against various stresses, like heat, and provide the bacterium with a chance to survive extreme circumstances. *B. cereus* is such a pathogen and is associated with cereals. It's spores sporulate when kept at 30-37 degrees Celsius for too long, but growth has even been observed around 15 °C and 43 °C (Gilbert et al., 1974). This could pose a problem during the often slow cooling-down step, especially in the production of bigger quantities of munkoyo/chibwantu.

Resilience of munkoyo/chibwantu against invasion

As mentioned in the main introduction, fermented foods have several ways to resist pathogen invasion. Next to the mentioned properties, munkoyo/chibwantu is usually produced using a process known as backslopping. By reusing the same calabash or bucket, the microorganisms in the fermentation vessels are transferred from batch to batch and may adapt over time. This way, a relatively specialized and high initial inoculum is provided to start the fermentation, which speeds up fermentation and thus reduces the chance for pathogens to grow to significant numbers before fermentation is finished.

Another reason to suspect that the products could be resilient against invasion of pathogens is based on past research. Survival of pathogens in similar products to munkoyo/chibwantu has been tested by Simango and Rukure (1992). They found that *Aeromonas* and *Campylobacter* were eliminated almost directly after inoculation in mahewu, a South-African fermented millet drink. *Salmonella* was no longer detected after 4 hours. *Shigella* and *E. coli* were stayed alive for longer, but then also decreased sharply. Previous research specifically done on chibwantu and munkoyo provides further evidence by showing elimination of *E. coli, Shigella* and *Salmonella* during fermentation, but also during storage in the finished product. *S. aureus* did survive fermentation, but reduced sharply and did not survive storage in the fermented product (Mwale, 2014).

Previous research shows that fermenting microbes in munkoyo/chibwantu mainly belong to the *Lactobacillus* and *Weisella* genera (Schoustra, 2013). Most *Lactobacilli* are homofermentative and mainly produces lactic acid. Some are heterofermentative and thus also produce ethanol and CO_2

next to lactic acid. Most species of the genus *Weisella* are heterofermentative. In some samples species belonging to the genus *Acetobacter* have been found, which can convert ethanol in acetic acid if oxygen is available (Schoustra et al. , 2013).

Even if the product is resilient against invasion of pathogens, contamination of the product can still occur, for example during sales. Munkoyo/chibwantu are often sold at local markets from a big vessel. Customers either bring their own packaging or use a bottle or bag from the supplier. The beverages can therefore be considered street foods, as they are usually not sold pre-bottled in a supermarket. A paper on prevalence of food borne pathogens in street foods (including fermented foods) from seven African countries was done between 2000 and 2015 (Paudyal et al., 2017) and showed that these products are often contaminated with pathogens. The authors reported Enterobacteriaceae, *E. coli, Salmonella, S. aureus and L. monocytogenes* as the most prevalent pathogens in food. The authors found *E. coli* in 31.6% of the sampled street foods. *Salmonella* was found in 21.7% of the samples, *S. aureus* in 25,1 % and *L. monocytogenes* in 6.7% of the samples. 34.2% of the food samples was contaminated in a way.

In this chapter the survival of selected pathogens during fermentation in unused fermentation vessels and during storage was investigated. Furthermore samples of munkoyo/chibwantu were taken from Zambia and tested for presence of pathogens to determine how often samples are actually contaminated. The sampling locations consisted of markets, farms, people's homes and supermarkets. Fermentation was done in unused vessels

Materials and methods

Invasion experiments

Munkoyo preparation

Munkoyo was prepared in a cooking pot on an electric hot plate. Munkoyo was cooked as follows. One (1) liter of water was heated till 60 °C. 100 grams of mealie meal (coarse maize flour) was added spoon by spoon and stirred in between to avoid lump formation. The mixture was then brought to a boil and kept boiling for 20 minutes until the white foam that forms on top disappeared. The mixture was then cooled to 45 °C. 140 grams of water was boiled for 5 minutes and then cooled back to 30 °C. 10 grams of munkoyo root was soaked in the lukewarm water for 50 minutes. The roots were then removed and the extract was added to the cooled porridge.

The mixture was then divided over 100 ml Schott flasks. The caps were unscrewed slightly to allow some oxygen into the flasks. The munkoyo was fermented for four days at 25°C with or without added pathogen.

Pathogen preparation

Each culture of a pathogenic bacterium was first streaked from a freezer stock culture onto Brain Heart Infusion Agar (see table 1 for specifications). After 24 hours of growth on the BHI-agar, one colony of each pathogen was taken and inoculated in LB-broth. The broths were incubated aerobically over night at 37 °C. It was assumed that the broth contained approximately 9 log CFU/ml after the overnight incubation. 1 ml of the overnight broth was taken and centrifuged at 10000 rpm for 5 minutes. The supernatant was discarded. The pellet was resuspended in 1 ml PFZ. 100 µl of this suspension was used to spike the Schott flasks containing 100 ml of fresh munkoyo to obtain an approximate spiking rate of log 6 CFU/ml.

Pathogen	**Strain**	**Selective agar for pathogen detection**	**Incubation temperature and time**
S. aureus	DSM799, Sc0108	Mannitol Salt Agar (OXOID)	37 °C; 24 h
L. monocytogenes	Scott A, Li0001	Brilliance Listeria Agar (Fisher Scientific)	
S. enterica	DSMZ 9587, Sa0222	Brilliance Salmonella Agar (Fisher Scientific)	
E. coli	K12	MacConkey No. 3 (OXOID)	
B. cereus	DSM 345, Ba0076	Mannitol Egg Yolk Polymyxin Agar (Fisher Scientific)	30 °C; 24 h

Table 1 List of pathogens and their propagation specifications

Survival of pathogens during fermentation

For the invasion during fermentation munkoyo was prepared. At the start of fermentation (t=0) each bottle was spiked with one of the pathogens. This was done in triplicate. The munkoyo was incubated at 25 °C and fermentation took 4 days. For *E. coli* and *S. aureus* samples were taken every 24 hours up to 96 hours. The remaining three pathogens were only sampled after 96 hours. At each time point taken, the pH and the number and/or presence of the pathogen were determined by

spiral plating the -1 and -4 dilution of the munkoyo on the corresponding selective agars (see table 1).

Invasion of pathogens during storage

Each pathogen was spiked into 96 hour fermented munkoyo in triplicate. The munkoyo was put in the refrigerator at 7 °C. Samples were taken after 48 and 96 hours to check the pH and number and/or presence of the pathogens by spiral plating the -1 and -4 dilution of the munkoyo on the corresponding selective agars.

Detection of pathogens from Munkoyo/chibwantu samples

Munkoyo/chibwantu sampling

Each producer filled three 50 ml screw cap tubes with munkoyo or chibwantu. The samples were kept refrigerated at 7 °C. 1 tube was used for culture-based pathogen detection and pH measurements. The remaining tubes were frozen and kept at -18 °C for further analysis in the Netherlands.

DNA extraction munkoyo/chibwantu

1 gram of product was taken. Large particles were avoided by pipetting carefully. The product was spun down for 2 minutes at 13000 rpm. The supernatant was removed. 500 µl TESL (25mM Tris, 10 mM EDTA, 20% sucrose, 20 mg/ml lysozyme (Merck), 10 µl mutanolysin (1 U/µl) solution and 100 µl lysozyme solution (10 mg/ml) was added. The tube was vortexed and incubated at 37 °C for 60 minutes with slight shaking (300 rpm). 500 µl GES reagent (5M guanidium thiocyanate, 100 mM EDTA, 0.5% sarkosyl) was added and the tube was then kept on ice for 5 minutes. 250 µl of cold ammonium acetate solution (7.5 M) was added and the two phases were gently mixed. The tube was kept on ice for 10 minutes and then spun down for 2 min at 13000 rpm. To purify the samples, 750 µl of the upper layer was taken and mixed with 750 µl chloroform-2-pentanol (24:1). The samples were vortexed vigorously and subsequently spun down for 5 min at 13000 rpm. 600 µl of the upper layer was taken and mixed with 600 µl phenol (tris-saturated Phenol-Chloroform-Isoamylethanol 24:25:1), vortexed and spun down for 1 min at 13000 rpm. 500 µl of the upper layer was taken and mixed with 500 µl chloroform, vortexed and spun down for 1 min at 13000 rpm. 300 µl of the upper layer was taken and precipitated using 300 µl ice cold isopropanol. The samples were put away at -20 °C overnight. The next day the samples were spun down for 15 min at 13000 rpm. The supernatant was discarded carefully and the samples were washed with 70% ethanol without vortexing and spun down for 2 min at 13000 rpm. The ethanol was carefully aspirated and the samples were left to air-dry for 10 minutes. The DNA was then dissolved in 50 µl TE buffer (10 mM Tris, bring to pH 8.0 with HCl; 1 mM EDTA) and incubated at 37 °C for 30 minutes. The DNA was kept in the freezer at -20 °C. Nanodrop was used to indicate the amount of DNA present and a 1% agarose gel with 0.005% ethylbromide was used to check if the extraction had been successful.

Preparation of the samples

25 grams of product was poured into 225 ml of Buffered Peptone Water (BPW, Oxoid). The mixture was left at room temperature for 1 hour. Ringer's solution was subsequently used to further dilute the samples.

Staphylococcus aureus

S. aureus was detected and enumerated using Baird-Parker Agar (BP-agar, Oxoid) supplemented with Egg Yolk Tellurite Emulsion (5% v/v). Using the drip technique (Herigstad et al., 2001) 20 µl of adequate dilutions where pipetted onto the agar. The agar was subsequently incubated at 35 °C for 48 hours. Suspect *S. aureus* colonies were counted at 24 and 48 hours. To confirm that they were indeed *S. aureus*, they were examined under the microscope.

Bacillus cereus

B. cereus was detected and enumerated using Mannitol Yolk Polymyxin agar (MYP-agar, Oxoid) supplemented with Egg Yolk Emulsion (5% v/v) and Polymyxin B Supplement. Using the drip technique (Herigstad et al., 2001) 20 µl of adequate dilutions where pipette onto the agar. The agar was subsequently incubated at 30 °C for 24 hours. Typical *B. cereus* colonies were counted.

Salmonella and shigella

For detection of *Salmonella* and *Shigella* the BPW-sample mixture was incubated at 35 °C for 24 hours. 100 µl of the overnight broth was pipetted into 10 ml Rappaport Vassiliadis broth (RV-broth), which was subsequently incubated at 42 °C for 48 hours. To obtain single colonies, a drop of the broth was streaked onto both Xylose Lysine Deoxycholate agar (XLD, Oxoid) and Brilliant Green Agar (BGA, Oxoid). Both agars were incubated at 35 °C for 24 hours. Suspect colonies were then streaked onto and stabbed into Triple Sugar Iron agar (TSI agar, Oxoid) slants for confirmation. Next to that they were streaked onto Salmonella Shigella agar (SS agar, Oxoid). Both agars were incubated at 35 °C for 24 hours and subsequently checked for characteristic reactions/colonies.

Enterobacteriaceae

Enterobacteriaceae were detected using VRBG-agar (full name, Oxoid). 1 ml of the appropriate dilutions was pour plated and subsequently covered with a layer of agar. The plates were incubated at 35 °C for 24 hours. Suspect colonies were counted.

DNA extraction positive controls

DNA extraction for positive controls for PCR was done by inoculating each pathogen of which a positive control was available into LB-broth that was incubated overnight at 37 C. The pathogens that were available were *S. aureus, E. coli, Shigella, B. cereus, S. enteric* and *L. monocytogenes*. The DNA was extracted using the Wizard® Genomic DNA Purification Kit: "Isolating Genomic DNA from Gram Positive and Gram Negative Bacteria" (ProMega Co., Madison, WI, USA). Since there was no lysostaphin available 20 µl of the lysozyme solution was replaced with by 20 µl mutanolysin (4 U/µl, Merck, Darmstadt, Germany) for the gram-positive bacteria. Furthermore *S. aureus* was treated with micro beads for 30 seconds.

PCR munkoyo

2 µl of each DNA-sample was added to 23 µl of PCR mixture containing 50 mM Tris-HCl (pH 8.5), 20 mM KCl, 3 mM $MgCl_2$, 0.05% bovine serum albumin (GIBCO BRL life technologies), 0.25 mM of each of dATP, dTTP, dCTP and dGTP, 0.25 µM of each primer and 0.9 U GoTaq polymerase (promega Benelux BV, Leiden, the Netherlands). The PCR was conducted in a Biorad T100 Thermal Cycler. The amplification condition was one cycle of 94 °C for 15 seconds, then 35 cycles of 94 °C for 3 seconds, 50 °C for 10 seconds and 74 °C for 35 seconds at the maximum ramp rate and finally one cycle of 74 °C for 2 minutes and 45 °C for 2 seconds. The PCR products (5 µl of each) were dyed using 6x concentrated DNA loading dye (Thermo Scientific, Netherlands) and then separated by

electrophoresis in 1% agarose gels containing 0.005% ethidium bromide. The full codes of the primers used can be found in table 2 of the article of Wang et al. (Wang et al., 1997). The pathogens that were checked for were *E. coli, E. coli*-O157:H7, *Shigella* spp., *Salmonella* spp., *V. cholera, L. monocytogenes* and *B. cereus.* The primers were ordered at Biolegio BV (Nijmegen, Netherlands), dissolved in RNAase/DNAase-free milliQ water and kept at -20 °C.

Results and discussion

Survival of pathogens during fermentation

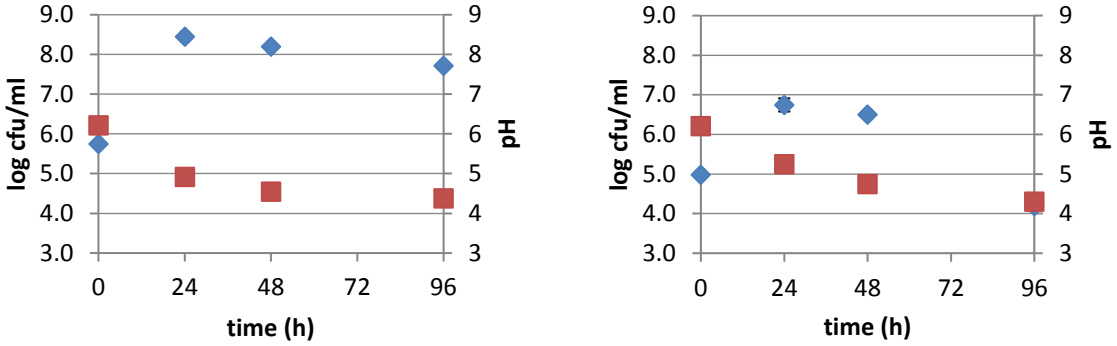


Figure 2 Number of *E.coli* (◆, left) and *S. aureus* (◆, right) in log CFU/ml and the pH ■) over time during fermentation of munkoyo (three replicates)

The number of *E. coli* increased from 5.7 log CFU/ml to 8.4 ± 0.4 log CFU/ml during the first 24 hours of fermentation. The number decreased to 7.7 ± 0.0 CFU/ml over the remaining fermentation time. The pH went down from 6.21 to 4.37 ± 0.08 over the entire fermentation. The microbial community of the munkoyo was not resilient against the invasion of *E. coli*.

The number of *S. aureus* increased from 5.0 log CFU/ml to 6.7 ± 0.2 log CFU/ml during the first 24 hours of fermentation. The number decreased to 4.2 ± 0.1 CFU/ml over the remaining fermentation time. The pH went down from 6.21 to 4.31 ± 0.06 over the entire fermentation. The microbial community of the munkoyo was not resilient against the invasion of *S. aureus*.

It was expected that *E. coli* would no longer be detected at the end of fermentation, as was demonstrated in previous research done on chibwantu (Mwale, 2014). Various explanations could explain the obtained results of our study. Samples were taken until the pH remained stable (pH = 4.5), which took 96 hours. This value was comparable to levels found in chibwantu bought from street vendors (4.0-4.5), but was still 0.5 higher than for munkoyo samples from street vendors (3.5-4.0) (Schoustra et al., 2013). *E. coli* has a growth limit at pH 4.5 (Small et al., 1994), which explains the initial growth and slight decrease from t=48, where the pH is 4.8. A pH of 4.5 is still high enough to survive though for most pathogens, which correlates to the remaining presence of *E. coli*. Furthermore, slow acidification can trigger pathogens to adapt to acidic circumstances using a variety of acid-resistance systems (Foster, 2004).

Mwale also suggested that it is not the pH, but the munkoyo root species that is responsible for removing *E. coli*. Both roots used by her removed *E. coli* within 24 hours. When using a yellow root the pH did not get lower than 5.63. Also, the amount of *E. coli* in the yellow root-munkoyo even

reduced more rapid than when using a white root. Both roots used in her research actually stimulated growth of *E. coli* in munkoyo root extract that was not mixed with the maize porridge, which again would disprove that the roots would contain inhibitory substances towards *E. coli*. Throughout Zambia different roots are used, which makes it hard to indicate which root should be used to have an inhibiting effect on *E. coli.* Further research on the different plant species should be performed to figure out if there are significant differences between the different used species.

The low acidification rate could furthermore indicate a slower growth of the microbial community of the munkoyo and therefore less competition for the pathogens, giving them more time to adapt. A lower growth rate could be caused due to differences in the ratio munkoyo root:maize:water. In the research performed by Mwale, this ratio was 2.5:15:100, whereas the ratio in this experiment was 1:10:100. In this experiment there was thus less substrate and probably also less enzyme to break down the starch in the porridge. Furthermore it is not described if the fermentation bottles used by Mwale were sterile. If they were not, fermentation might have started sooner than in the sterile bottles used in this experiment.

In the research of Mwale *S. aureus* was still detected during fermentation up to a final pH of 4.31 at a rate of 6.08 log CFU/ml. However the final pH is similar to this experiment, *S. aureus* reduced further than in her experiment. In her experiment *S. aureus* was still found at the end of fermentation. However the decrease of the pathogen was stronger when the yellow root was used. She also showed that *S. aureus* cannot grow in munkoyo root extract, which could explain the strong decrease of *S. aureus* compared to *E. coli.*

Invasion of pathogens during storage

			pH				log CFU/ml			
		Time (days)	Fermentation control	Fermentation with pathogen	Storage control	Storage with pathogen	Fermentation control	Fermentation with pathogen	Storage control	Storage with pathogen (inoculation at fermentation t=4)
E. coli	**Fermentation**	0	6,21	6,21	6,21	6,21	<1	5,75		
		4	4,25	4,37 ± 0,08	4,57	4,57	<1	7,71 ± 0,02	<1	6,15
	Storage	2			4,53	4,44 ± 0,13			<1	5,70 ± 0,09
		4			4,46	4,30 ± 0,20			<1	5,62 ± 0,18
S. aureus	**Fermentation**	0	6,21	6,21	6,21	6,21	3,83	4,98		
		4	4,25	4,31 ± 0,06	4,57	4,57	3,81	4,19 ± 0,13	3,81	6,57
	Storage	2			4,53	4,36 ± 0,17			2,90	3,71 ± 0,30
		4			4,46	4,51 ± 0,04			<1	3,69 ± 0,19
B. cereus	**Fermentation**	0	6,21	6,21	6,21	6,21	<1	7,05		
		4	4,57	4,24 ± 0,10	4,57	4,57	<1	5,82 ± 0,20	<1	6,75
	Storage	2			4,53	4,34 ± 0,07			<1	6,64 ± 0,07
		4			4,46	4,11 ± 0,39			<1	5,91 ± 1,42
Salmonella	**Fermentation**	0	6,21	6,21	6,21	6,21	<1	6,91		
		4	4,57	4,49 ± 0,07	4,57	4,57	<1	7,96 ± 0,23	<1	6,80
	Storage	2			4,53	4,40 ± 0,35			<1	5,82 ± 0,05
		4			4,46	4,41 ± 0,13			<1	5,41 ± 0,09
L. monocytogenes	**Fermentation**	0	6,21	6,21	6,21	6,21	<1	6,74		
		4	4,57	4,56 ± 0,05	4,57	4,57	<1	7,90 ± 0,33	<1	6,78
	Storage	2			4,53	4,45 ± 0,09			3,58	5,27 ± 0,53
		4			4,46	4,24 ± 0,26			3,08	4,67 ± 0,20

Table 2 Log CFU/ml of pathogens and the corresponding pH in spiked munkoyo during fermentation at 25 ˚C and in fermented munkoyo during storage for 4 days at 4 ˚C

E. coli

The survival of *E. coli* during fermentation was already discussed previously. Viable counts of *E. coli* reduced slightly from 6.1 log CFU/ml to 5.62 ± 0.18 over four days of storage. The pH went down from 4.57 to 4.30 ± 0.20. The microbial community was not resilient towards invasion of *E. coli* during storage. Based on previous research on chibwantu it was expected that *E. coli* would not be detected anymore after storage, where *E. coli* was already undetected after 24 hours of storage at a pH of 5.83, a pH at which *E. coli* is still capable to grow (Small et al., 1994). This could be due to antimicrobial compounds in the munkoyo roots used by Mwale or by anti-microbial compounds produced by the microbial community. In the storage experiment, *E. coli* did not endure a gradual increase in acidity, giving it less time to activate any acid resistance mechanisms. Similar results have been found for *E. coli* inoculated into beer that had a pH of 4.3 (Menz et al., 2011). It might be that the *E. coli*-strain used in this thesis is more resistant towards acids than the strain used by Mwale or that the microbial community did not produce (sufficient) antimicrobial compounds.

S. aureus

The survival of *S. aureus* during fermentation was already discussed previously. During storage the viable plate count of *S. aureus* drops from 6.57 log CFU/ml to 3.69 log CFU/ml over four days. The pH remained stable during storage. *S. aureus* was also found in the control sample. The munkoyo roots could be the source of those cells. *S. aureus* has been reported to survive for months on dry surfaces (Kramer et al., 2006), making it likely for the pathogen to survive on the dried munkoyo roots as well. Even if the numbers that were found in the control are subtracted from the numbers found in the spiked samples, *S. aureus* would still be found present after both fermentation and storage. The microbial community was thus not resilient towards invasion of *S. aureus* during storage.

In previous research *S. aureus*, was no longer detected after four days of storage. The pH-values at which they were no longer detected were 4.87 and 4.31 and depended on the munkoyo root used (Mwale, 2014). In previous research on the growth boundaries of *S. aureus,* growth was still observed at a pH of 4.5 at a temperature of 13 ˚C (Valero et al., 2009). It is therefore unlikely that it was the low pH that killed the *S. aureus* in the experiment of Mwale.

B. cereus

B. cereus dropped by approximately 1 log CFU/ml both during fermentation and storage. However, the standard deviation is 1.42 log CFU/ml. This does not make it possible to draw any conclusions about the exact rate at which the pathogen is killed. The microbial community was not resilient towards invasion of *B. cereus* during both fermentation and storage.

The pH dropped from 6.21 to 4.24 ± 0,10 during the fermentation process. During storage the pH dropped from 4.57 to 4.11 ± 0.39 over four days, which is 0.35 lower than for the control sample. The extra decrease in pH could be due production of acids by the pathogen self, since the circumstances in the bottom of the fermentation bottle are possibly anaerobic. *B. cereus* is able to ferment various carbohydrates by mixed acid fermentation in absence of oxygen (Ouhid-Jacobs et al., 2009). Even though there is a big standard deviation within the triplicate, there is no relation ($p < 0.05$) between a lower pH and the log CFU/ml within the triplicate.

In former research, cold shock response has been observed in *B. cereus* (Mayr et al., 1996). Also, *B. cereus* can grow up to a pH of 4.3 (Food Safety Authority of Ireland, 2016) in some food products.

Both characteristics make it likely for *B. cereus* to survive the munkoyo fermentation and storage if there are no other antimicrobial compounds present.

Salmonella

Salmonella spp. grew by approximately 1 log CFU/ml during fermentation. During storage, a reduction of approximately 1.4 log CFU/ml was observed. The microbial community was not resilient towards invasion of *Salmonella* during both fermentation and storage. The pH was comparable to the pH of the control sample. Based on previous research on chibwantu, *Salmonella* would not have been expected after four days of fermentation. However, in former research done on the survival of *Salmonella* in beer at a pH of 4.3, *Salmonella* did survive storage in cold beer over a prolonged period (Menz et al., 2011).

L. monocytogenes

L. monocytogenes grew by approximately 1 log CFU/ml during fermentation, but reduced by approximately 2 log CFU/ml during storage. The microbial community was not resilient towards invasion of *L. monocytogenes* during both fermentation and storage. The pH during fermentation was similar to the control sample. During storage the pH of some of the samples decreased more than the storage sample, but since there is only one control sample and the standard deviation within the triplicate was high (0.26), it cannot be concluded if the decrease was significantly stronger. *L. monocytogenes* is known to be very acid resistant. In presence of glucose it can even tolerate pH levels of 3.5 (Koutsoumanis et al., 2003). Furthermore survival was actually shown to be greatest at the low temperature of 10 ˚C (Kent M. Sorrells, 1990). Therefore it is not surprising that the pathogen survived fermentation and storage at low temperature. As can be read later on in chapter 2, sugars, most samples still contain glucose, increasing the survivability of *L. monocytogenes.*

Detection of pathogens from Munkoyo/chibwantu samples

		Positively tested			Log CFU/ml				
	pH	*S. aureus*	*B. cereus*	*Shigella & Salmonella*	Entero-bacteriaceae	TVC	Lacto-bacilli	Lactic streptococci	Yeast and moulds
Not finished fermenting	5,14 ± 0,04	1/1	1/1	0/1	1,7 ± 0	7,7 ± 0,0	8,3 ± 0	> 6,5 ± 0,0	3,1 ± 0
Just fermented	3,83 ± 0,3	3/7	2/7	0/7	3,4 ± 1,0	8,0 ± 1,0	8,0 ± 0,6	7,4 ± 1,0	5,4 ± 0,4
Kept at room T	3,30 ± 0,2	1/4	2/4	0/4	3,4 ± 1,5	6,9 ± 0,8	7,8 ± 0,7	6,1 ± 1,2	5,3 ± 0,7
Kept in cold storage	3,51 ± 0,1	2/4	1/4	0/4	2,4 ± 0,5	7,4 ± 0,0	7,3 ± 1,0	5,7 ± 0,8	4,9 ± 1,6

Table 3 Summary of pathogen presence in samples taken in Zambia, based on culture based methods.

In table 3 a summary of the results of the sample testing in Zambia are shown. The full table (table 4) can be found in appendix A. The different samples are classified on their age, rather than on which type of munkoyo/chibwantu they are. It can be seen that the older the sample is, the lower the pH is. The average pH between the groups was significantly ($p < 0.05$, ANOVA) different. Each group contained samples that were infected with either *S. aureus* (7/16), *B. cereus* (6/16) or Enterobacteriaceae (15/16). There is no relationship ($p > 0.05$) between the age of the sample and the amount of contaminations. None of the samples was contaminated with *Shigella* or *Salmonella* spp. There was no sample without a contamination.

The infective dose for *S. aureus* (10^5-10^6 cells) to produce enough toxins is reached in all samples (Schmid-Hempel & Frank, 2007). The pH, temperature and oxygen level determine if these toxins are actually produced. For example Smith et al. 1983 found that *S. aureus* produces enterotoxin at a pH of 4 and under aerobic circumstances, but a pH of 4.6 was needed when grown anaerobically. Other research showed that the majority of *S. aureus* strains do not produce detectable amount of enterotoxins at a pH below 5.1 and are even incapable to produce enterotoxin at a pH of 5.7 if the circumstances are anaerobic (Smith, Buchanan, & Palumbo, 1983; Tatini, 1973). The circumstances in munkoyo vary throughout the fermentation container from anaerobically in the bottom of the container to aerobically in the upper part of the container, therefore *S. aureus* is possibly not able to produce enough toxins before the end of fermentation. This is also reflected in chapter 3, where it is shown that vomiting is rarely experienced after drinking munkoyo/chibwantu. Also, if the sample was first contaminated after fermentation, it is even more unlikely that toxins will be produced due to the low pH. Contamination can happen via the addition of the root or during handling of the product (e.g. filling of the bottles). The data from chapter 3, the quantitative producer questionnaires, was linked to the presence of these pathogens. 11 out of 13 producers reports to wash their hands before production or handling, however, 6 of them still have *S. aureus* in their product. Also taking off jewellery did not seem to matter, since 4 out of 5 that take of their jewellery still contain *S. aureus*. Cleaning by hand or using water was not removing the pathogen either. Furthermore there is no relation ($p > 0.05$) between sieving and cooling or age of the finished product and contamination.

The viable plate counts found for *B. cereus* are potentially enough to cause diarrhoea (at least 10^5-10^7 cells taken in) in 3 out of 6 samples. 2 out of 6 samples have the potential to produce enough toxin to cause vomiting (10^5-10^8 cells per gram) (Rolain & Raoult, 2006). To produce enterotoxin the pH needs to be above 5.0 (van Netten et al., 1990). The fermentation of most producers (10 out of 13) is finished fast (8 hours) which makes it unlikely that a lot of enterotoxin has been produced during fermentation. However, to reduce the risk of growth of *B. cereus* as much as possible, it is still important that the maize porridge is cooled down as quick as possible, so that potential spores cannot sporulate. The spores could also be boiled to death by boiling the porridge long enough. The D-value (time need to reduce number of pathogens by 1 log at a certain temperature, noted as $D_{temperature}$ = x min.) of *B. cereus* spores varies per product. 4.2 min. has been reported in rice broth of 100 ˚C, but 40 minutes are needed in pumpkin pie of 100 ˚C (Food Safety Authority of Ireland, 2016). Even lower values of 2.7-3.1 have been reported by Kramer and Gilbert (Kramer et al., 2006). The $D_{100°C}$ of *B. cereus* in munkoyo should be determined to know how long the porridge needs to be boiled to eliminate all spores. The presence of *B. cereus* could not be related to the fermentation time. Furthermore there is no correlation ($p > 0.05$) between or age of the finished product and contamination.

Using PCR (see figure 6, appendix A), no pathogens were detected in any of the samples obtained from producers. *B. cereus* is known to be resistant to lysozyme levels op to 100 µg ml^{-l} (Hughes, 1971) and to have a high resistance against mutanolysin (Raddadi et al., 2004). The concentrations of lysozyme used for the munkoyo DNA-extraction were much higher: 20 mg ml^{-l}, but apparently this was not sufficient. *S. aureus* and *L. monocytogenes* are also resistant to lysozyme (Bera et al., 2006; Burke et al., 2014). To make sure that the DNA of *S. aureus* is also extracted lysostaphin could be added (Wu et al., 2003). The protocol for DNA-extraction of munkoyo should be adapted to make sure that the DNA of these pathogens is also extracted. *E. coli* was also not detected using PCR. This could indicate that none of the found Enterobacteriaceae are *E. coli.* Since almost all samples were contaminated with Enterobacteriaceae it is not possible to relate any of the mentioned hygienic measures to these contaminations.

All samples that finished fermenting had a pH below 4. In previous research a pH between 3.5-4.0 was observed for munkoyo and between 4.0-4.5 for chibwantu (Schoustra et al., 2013). In this experiment there was no correlation ($p > 0.05$) between the pH and the fermentation container used. Recommended is to have a product with a pH below 4, since most pathogens cannot grow under those circumstances (webRFA, 2005).

Conclusion

The microbial community in munkoyo/chibwantu is not resilient against invasion of pathogens neither during fermentation nor during storage. When compared to previous research, it seems that the survivability of the selected pathogens is related rather to the fermentation time and the munkoyo root used than to the pH. *S. aureus* does decrease by 0.8 ± 0.1 log CFU/ml during the course of the fermentation process and by 2.9 log CFU/ml during four days of storage at 7 °C. *B. cereus* does decrease by 1.23 log CFU/ml over fermentation. *E. coli* reduces by 0.5 log CFU/ml over storage, *Salmonella* by 1.4 log CFU/ml and *L. monocytogenes* by 2.11 log CFU/ml.

15 out of 16 samples obtained from producers were contaminated with *S. aureus, B. cereus* or Enterobacteriaceae. All samples contaminated with *S. aureus* contained the infective dose. For *B. cereus* only half of the samples contained the infective dose. However, for all samples the pH was probably too low to cause any adverse effects.

Chapter 2: compounds that influence the safety of munkoyo/chibwantu

Introduction

Not only microbes can pose a risk to munkoyo/chibwantu. During production various compounds are produced or introduced. These can reduce the safety of the product or even be dangerous. This chapter starts with a short literature review on a selection of those compounds (acids, sugars, ethanol, munkoyo root, acrylamide and mycotoxins). Furthermore, samples that were taken from the field were analyzed on their ethanol, sugar and acids content. Other (toxic) compounds were not tested for, but it is strongly recommended to do so in the future to obtain a better insight in the safety of the product.

Acids and sugars

Acids and sugars may not pose a hazard to one's health, but they do influence the overall microbial stability of the product. For sugar to work as a preservative, the contents need to be very high, such that the water activity in the product is lowered to such an extent that bacteria can no longer grow in the substance. This is not the case for munkoyo/chibwantu. Remaining or added sugars can actually reduce the shelf life of the product by providing substrate for pathogens or increase their acid resistance, as was for example shown for *L. monocytogenes* (Koutsoumanis et al., 2003).

Several acids can be produced during fermentation, like lactic acid, acetic acid or succinic acid. Lactic acid is usually the most abundant. The values of lactic acid in munkoyo/chibwantu are expected to lay around (1.35% (w/v)) based on values that were found in previous research on the similar product mahewu (Fadahunsi & Soremekun, 2017). Acids lower the pH of a product and thus inhibit or even eliminate pathogens in foods. The lower the pH, the higher the antimicrobial capability of the acid, since the acid preserves better when un-dissociated. Also, it has been found that a mixture of acetic acid and lactic acid works even more inhibiting than the sole acids (Adams & Hall, 1988). The toxicity of fermentation acids is explained by the transmembrane flux of undissociated acids, proton release in the alkaline interior of bacterial cells and the dissipation of the proton motive force. However, there is also research that shows that some pathogens can have several mechanisms against the inhibitory effects of fermentation acids. An example is the use of anti-porters to pump out H^+ from the cell (Foster, 2004; Gorden & Small, 1993; Small et al., 1994). Even though acids can contribute to the safety of a product, fermentation should not be relied on as a means of removing pathogens. Food production should always be hygienic and initial levels of pathogens and toxins should be as low as possible.

Ethanol

If munkoyo ferments for longer it turns alcoholic (some now call the drink chibuku, others discard it), and is usually no longer served to children. However, during this transition there is a gray zone in which the product already contains ethanol, but is still served to children. It is important to know how much ethanol is in munkoyo to determine if this poses a hazard to children and if the production method influences the ethanol content. If the product will be produced on a larger scale it should be free from alcohol so that it can be sold to all ages.

Munkoyo root

Throughout Zambia different roots are used to prepare munkoyo. Some people even use flour or the peels of sweet potatoes. Each producer has his or her own theory about which roots to use, but they all agree that one needs to be careful when picking the root, since some can be poisonous. Munkoyo roots have been so far assigned to belong to the genera *Eminia*, *Rhynchosia* or *Vigna* (Foma et al., 2013) and suggestions have even been done on where to cultivate the roots in case one wants to upscale the production of munkoyo (Ergo, A.B. et al., 1994). It is unknown which plants are the ones that are confused with the real munkoyo root and what makes them toxic.

Acrylamide

When starchy foods, that contain asparagine and reducing sugars like glucose and fructose, are burnt, acrylamide can be formed through the Maillard-reaction. Maize does contain asparagine and has been shown to be able to contain acrylamide after heating (Galani et al., 2017). It is still debatable if acrylamide in foods actually pose a risk (Cancer Research UK, 2016). The northern-type munkoyo is deliberately burnt. Some microorganisms, for example *B. cereus* have been found to break down acrylamide. *B. cereus* is unwanted in the fermentation of munkoyo/chibwantu, but other microorganisms could be selected or looked for. The fermentation could then possibly reduce the amount of acrylamide (Kusnin et al., 2015). This is not further tested in this thesis, but could be an interesting topic for future research.

Mycotoxins

Zambia's climate is warm most of the year and knows a rainy season around December. High humidity and heat are the perfect circumstances for fungi of the genera *Penicillium*, *Fusarium* and *Aspergillus* to grow on the maize used for munkoyo/chibwantu. These fungi may produce mycotoxins. If farmers and producers are not careful during harvesting and storage, the risk of mycotoxin production even increases (Chulze, 2010; Ruiz de Galarreta et al., 2015). Checking for the presence of mycotoxins in the raw material is not possible for producers at household level and is possibly even too expensive for slightly bigger producers. There are some indications that fermentation can break down certain mycotoxins (Ji, Fan, & Zhao, 2016; Valle-Algarra et al., 2009), however it is still better to prevent mycotoxin contamination. In this thesis there is no further testing done on the mycotoxin content of munkoyo/chibwantu, but these compounds also pose a hazard to the health of the consumer.

Materials and methods

Ethanol, acid and sugar detection

Munkoyo/chibwantu sampling

See chapter 1, “materials and methods”, munkoyo/chibwantu sampling.

HPLC

To quantify the amount of ethanol, acetic acid, formic acid, citric acid, lactic acid, glucose, fructose, mannitol, maltose and sucrose in the munkoyo, High Performance Liquid Chromatography (HPLC) was performed on an Ultimate 3000 HPLC (Dionex) equipped with an RI-101 refractive index detector (Shodex, Kawasaki, Japan), an auto sampler and an ion-exclusion Aminex HPX – 87H column (7.8 x 300 mm) with a guard column (Bio-Rad, Hercules, CA). The mobile phase was 5 mM H_2SO_4 and the flow rate was 0.6 ml/min at 40 ºC.

0.5 ml of each sample was mixed with 0.25 ml Carrez A and 0.25 ml Carrez B solution. This was vortexed well and then centrifuged at 13000 rpm for 5 minutes. 200 µl of the supernatant was subsequently put in HPLC-vials. A standard of the sugars with concentrations between 0-25 mM and alcohol concentrations ranging from 0-5% (v/v) and acid concentrations ranging from 0-50 mM were used to make a standard curve. To determine where each peak in the HPLC chromatogram is located, standards of the sole compounds were made. The injection volume was 10 µl. Total run time was 30 minutes. Output was analyzed using Chromeleon 7 Chromatography Data System. All samples were analyzed in duplicate. SPSS was used to look for significant differences, using a one-sided ANOVA to compare groups (based on product kind, microorganism content or processing variable) and a one sample t-test to look for significant differences within one product group. Pearson’s correlation was used to correlate microorganism abundance to certain compounds. In the appendix the used test per comparison and the corresponding p-values can be found.

Results and discussion

Acids

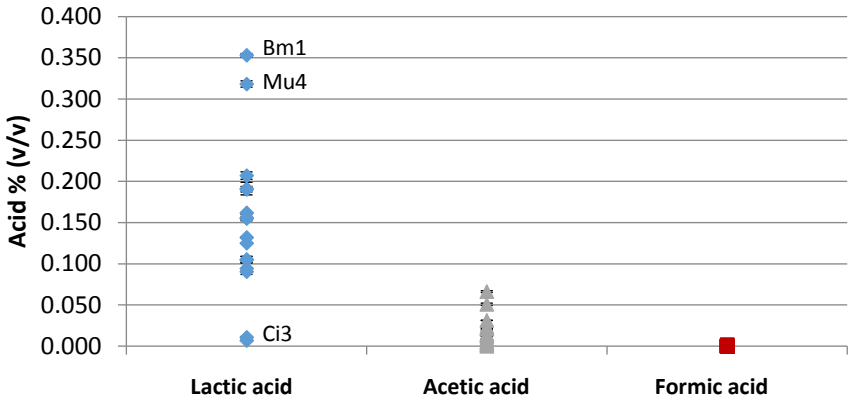


Figure 3 Acid concentrations % (v/v) of lactic acid (◆), acetic acid (▲) and formic acid (■) in munkoyo/chibwantu samples taken from Zambia (two replicates)

Lactic acid concentrations did not vary significantly between the product groups and varied from 0.007 ± 0.000 % (v/v) to 0.353 ± 0.002 % (v/v). Acetic acid concentrations varied significantly between groups ($p < 0.05$) from 0.000 ± 0.000 % (v/v) to 0.066 ± 0.001 % (v/v). Within chibwantu both lactic and acetic acid levels differed significantly ($p < 0.05$). Only two samples (Ci7 and Mu1) contained formic acid, but in small amounts (0.001 ± 0.000 % (v/v) and 0.002 ± 0.000 % (v/v) respectively). One sample (Bm2) contained 0.01 g/L citric acid (0.003 % (w/w)).

There is no significant difference ($p > 0.05$) between any of the acids and the various processing variables (the fermentation container, cooking time, use of root extract and age of the product. All values are non-continuous). The only correlation ($p < 0.05$) found between microorganisms and an acid was between enterobacteriaceae and acetic acid. Indicating they could be the reason for presence of acetic acid, which is likely since mixed-acid fermentation the fermentation pathway that occurs in enterobacteriaceae (Madigan et al., 2003). Furthermore heterolactic fermentation can have taken place in these samples. Heterolactic fermenters are *Leuconostoc mesenteroides, Lactobacillus bifermentous* and *Leuconostoc lactis* (Karki, 2017). For Ci6 only lactic acid was found and an ethanol amount that was close to 0, suggesting only lactic acid (homolactic) fermentation took place in this sample, which could be done by the species *Streptococcus thermophiles, Streptococcus lactis, Lactobacillus lactis, Lactobacillus bulgaricus, Pediococcus, Enterococcus*. Also the difference in acetic acid between the product groups was significant ($p < 0.05$) There is a correlation ($p < 0.05$) between the amount of formic acid and the moment of root addition, however, only two samples contained formic acid, so this can be a coincidence. Even though acids contribute to the microbial safety of a product, a lot of samples have been found to be contaminated (Chapter 1). As was discussed in chapter 1, the acids probably did inhibit growth of the pathogens, but due to acid-resistance or contamination later in the process the pathogens can still be found. The concentration of acids needed to preserve a product is variable and influenced by the pH and other factors like temperature or the use of other preservatives. The pH of all the finished samples was below 4.5. The pKa-values of lactic acid and acetic acid are 3.08 and 4.75 respectively, indicating that especially in the acetic acid will properly function as a preservative (Lücke et al., 1991).

Ethanol

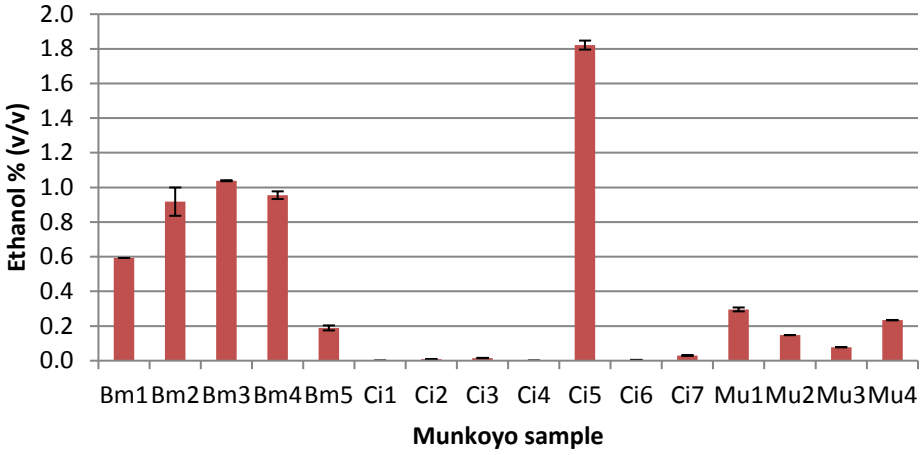


Figure 4 Average concentration % (v/v) of ethanol in fermented munkoyo taken from Zambia (two replicates). Bm = burnt munkoyo, Ci = chibwantu and Mu = munkoyo.

All chibwantu (Ci) samples expect for Ci5 contained less than 0.030 ± 0.001 % (v/v) ethanol. The values within the chibwantu samples did not differ significantly ($p > 0.05$), as long as Ci5 is left out of the comparison. Ci5 contained 1.822 ± 0.026 % (v/v) ethanol. The ethanol content of the northern-type munkoyo (Burnt munkoyo; Bm) samples varied from 0.189 ± 0.014 till 1.039 ± 0.003 % (v/v). Within the group of burnt munkoyo the samples also did not differ significantly ($p > 0.05$). The central/eastern type of Munkoyo (Munkoyo; Mu) contained between 0.148 ± 0.000 % (v/v) and 0.296 ± 0.011 % (v/v) ethanol. Within the group of munkoyo the amount of ethanol did not differ significantly ($p > 0.05$). Between the different categories of munkoyo the amount of ethanol did differ significantly ($p < 0.05$).

The only significant difference found in ethanol was between the product's raw materials and the ethanol content: for chibwantu coarser maize meal (maize grits) was used. There was no significant different ($p > 0.05$) between fermentation containers, cooking time, the use of root extract, the moment of the addition of the munkoyo root or the age of the munkoyo. There is a correlation ($p < 0.05$) between the amount of ethanol and the number of yeast and moulds (see table 4 in the appendix). Research has shown that co-cultures of yeast and bacteria could produce higher ethanol yields than bacteria or yeasts on their own, which could explain the higher amount of ethanol in the more yeast-rich samples (Laobussararak et al., 2012). There is no relation ($p > 0.05$) between the product type and the amount of any of the other microorganisms checked for.

Ci4 and ci5 are of the same producer, but ci5 remained refrigerated for 3 weeks before it was tested for pathogens and ethanol. It has 1.8 % (v/v) more ethanol. The amount of yeast and moulds is also higher (3 and 6.4 respectively, see chapter 1 "presence of pathogens"). The batches of which both samples originated were different, but this could indicate that fermentation continues during storage, even in the refrigerator. This would mean that the drink, when prepared without a controlled process, is only suitable for children for a certain period. All chibwantu (except for the old sample) and munkoyo samples are considered non-alcoholic, since their ethanol content is lower than 0.5 % v/v. Four out of five burnt munkoyo samples are considered low-alcoholic (<1.2 % v/v), and are therefore not suitable for consumption by children (Bránýik et al., 2012).

Another argument why the product's specific process would influence the amount of ethanol is given since Ci1 and Bm1 are from the same producer. The difference can be found in the process, thus: the raw material, cooking time, amount of munkoyo root and sieving or not. The cooking time did not correlate to the amount of ethanol. The sieving did, but that relates more to the size of the substrate than to the actual step of sieving, although a sieve could introduce microorganisms. The amount of munkoyo root added was not measured, but differed per producer (see chapter 3). This could also influence the ethanol content by providing more amylase that breaks down the starch into fermentable sugars.

Sugars

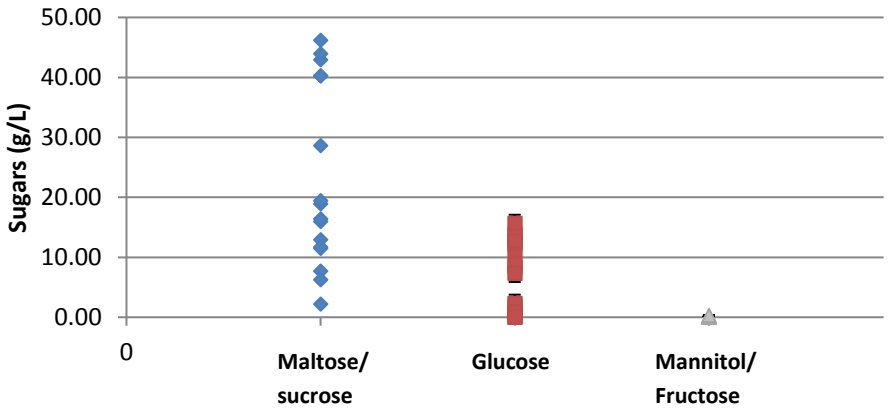


Figure 5 Sugars (g/L) in munkoyo: maltose/sucrose (◆), glucose (■) and mannitol/fructose (▲) in munkoyo/chibwantu samples taken from Zambia (two replicats)

Since sucrose and maltose and mannitol and fructose co-elute, the values were combined. Sucrose is added by some producers in varying amounts, making it unsure how much maltose is left after fermentation. The final concentration sucrose/maltose ranges from 2.2 ± 0.0 g/L to 43.16 ± 0.0 g/L. Glucose can better be used as an indicator to see if fermentation was completed, since no new glucose is added. Concentrations range from 0 .0 ± 0.0 g/L to 15.63 ± 0.0 g/L. For half of the samples, fermentation was practically completed (glucose <2.3 g/L), however there were still pathogens detected in those samples. Mannitol/fructose is only found in ci6 and mu4 at 0.03 ± 0.0 and 0.31 ± 0.0 g/L respectively. The amount of sugar in munkoyo/chibwantu is not enough to act as a preservative, but rather is an extra possible substrate for spoilage or pathogenic bacteria (FAO, 1995).

Conclusion

Ethanol can be found in all samples tested within a range of 0.03 ± 0.001 % (v/v) till 1.822 ± 0.026 % (v/v). There is a significant difference ($p < 0.05$) between the products and their ethanol content. Munkoyo and chibwantu are non-alcoholic and therefore suitable for children. Four out of five burnt munkoyo samples were low-alcoholic and should therefore be avoided by children.

Lactic acid was found in all samples and varied from 0.007 ± 0.000 % (v/v) to 0.353 ± 0.002 % (v/v). Acetic acid concentrations varied from 0.000 ± 0.000 % (v/v) to 0.066 ± 0.001 % (v/v). Only two samples contained formic acid (0.001 ± 0.000 % (v/v) and 0.002 ± 0.000 % (v/v) respectively) and one sample (Bm2) contained 0.003 % (w/w) citric acid. The acids lower the pH and function as an antibacterial compound at low pH. Therefore they contribute to the safety of the product.

Sucrose/maltose concentrations range from 2.2 ± 0.0 g/L to 43.16 ± 0.0 g/L. Glucose concentrations range from 0 .0 ± 0.0 g/L to 15.63 ± 0.0 g/L. Two samples contained mannitol/fructose at concentrations of 0.03 ± 0.0 and 0.31 ± 0.0 g/L respectively. These amounts do not contribute to preservation of the product and rather can serve as a substrate for pathogens or spoilage bacteria.

Chapter 3: risk perception of munkoyo/chibwantu of consumers and producers

Introduction

In 2009 162 people were hospitalized after drinking munkoyo (Lusaka Times, 2009) , one even died in 2013. 2014 had 2 deaths and 115 hospitalized (Kabaila, 2014; Allafrica News, 2014), 2016 hit 44 (Zambian Eye, 2016). In 2017 93 were hospitalized and one more person died (Zambia National Broadcasting Corporation, 2017; Zambia Daily Mail Limited, 2017). Symptoms varied per case from diarrhea and vomiting to dizziness and unconsciousness. In many cases it was suspected that either wrong munkoyo roots were used or that someone deliberately poisoned the crowd, however in some cases it was also suspected that is was due to food poisoning, which fits to the combination of the symptoms diarrhea and vomit. Interestingly, no such cases can be found for chibwantu, although the processes are fairly similar.

However many cases of illness after munkoyo consumption have occurred, previous research showed that local people claim that the product is safe and can actually offer health benefits. For example the prevention and cure of diarrhea. In short, there is a lot of conflicting information on munkoyo/chibwantu and the perception of its safety. No research has been done on actual occurrence of contamination by pathogens or on the possible toxicity of the munkoyo roots. Also, in cases where people got ill, the samples have so far not been checked on the actual reason for the illness. This always remained speculative.

There is a big informal food vending sector in Zambia. Hygienic practices at these food stalls are often not checked and water supply can be poor or of bad quality (Taulo et al., 2008). Furthermore, the knowledge on food safety by street vendors is not always sufficient and many people in Zambia (55,3 %) are illiterate, making it harder to spread knowledge on good hygiene or manufacturing practices (AdsumFoundation, n.d.). The rates of contamination of foods as mentioned in chapter 1 are alarmingly high and it is important to know how informed producers and consumers are, to see if they realize the risks of certain products and their preparation and so design educational documents if necessary. In this chapter interviews were done to determine the perception of risk and the measures that are now taken to avoid risks by both producers and consumers.

Materials and methods

Consumer demographics

120 consumers were interviewed systematically using the consumer questionnaire as found in appendix C. To ensure a diverse group of respondents, interviews were performed within Lusaka in malls, on various markets and at the UNZA, but also in Choma, Kabwe, Kitwe and surrounding villages. The data were analyzed using SPSS. The Fisher's exact test was used to see if there were any connections between answers given by the respondents.

The division of gender of the respondent was exactly 50/50. The majority of the respondents (41.7% was between 20 and 29 years old, followed by 30-39 (26.7%) and 40-49 (19.2%). 67.5% of the respondent were (self-)employed, 13.3% was studying, 10% was unemployed and 5.8% was farmer. Tonga and Bemba were the most represented tribes (27.5 and 21.7% respectively). Other abundant tribes were Tumbuka (7.5%), Chewa (5%) and Lala (5.8%). 45% of the respondents lived in the capital, 41.7% in smaller towns and 10.5% in villages.

Producer demographics

Producers were interviewed both qualitatively and quantitatively. The qualitative interview was only done with producers that had a larger-scale production (>50 liters per week). Three producers met this prerequisite. One extra producer that produced 30-50 L per week was interviewed since she showed interest in up scaling her production. The qualitative interview was done by using pre-made questions (appendix C) as a base for conversation. All response was written down during the interview and later combined into one story.

The quantitative questionnaire was performed on 13 producers and can be found in appendix C. The data was analyzed using SPSS. Of each producer one or two product samples were taken that were analyzed in the laboratory. 50% of the producers produced daily, 37.6% one to a couple of times per week, the rest produced less often. The majority (37.5%) produced between 30-50 L each time. 37.5% produces 15-30 L. 81.3% produces for commercial use, the rest for friends/family. 43.8% bottled their product themselves, 25% let the customer bring a bottle and the rest offered both options. Most producers sold their product on the market (42.1%) or from home (26.3%)

To make a first assessment of possible correlations between certain answers and groups (product type and process variables), histograms on the relative percentage of answers per group were made. Subsequently, a Fisher's Exact Test was performed to see if there were any associations between the groups and their answers.

Results and discussion

Quantitative consumer questionnaire

Overall outcome

The complete outcome of the questionnaire can be found in table 7 and 9 in the appendix. When asked if munkoyo is safe, 59,2% of the interviewed answered yes. 25% think it is, as long as one knows how to prepare it. Only 13,3% really thinks that it's not safe at all. 84.2% prefers traditional munkoyo over commercial varieties. From this it can be concluded that most people drink munkoyo with not too much suspicion; however trust was a frequently mentioned topic when people were asked where they obtained the product. Whenever they buy or get munkoyo, the Zambians like to know the person or source where it comes from. This is also reflected in the questionnaire: 27.6% makes the munkoyo themselves, 35.9% gets it from friends or family and 8.3% buys it from someone in the neighbourhood. Only 14.5% buys munkoyo at the local market. These high numbers are repeated when asked how they make sure that the munkoyo they drink is safe for consumption. 37.8% gets the munkoyo from a familiar source. 37 out of 40 that make the product themselves claim to do so because of safety reasons. Other frequently given answers to ensure a safe product are checking the product visually (14.8%) and checking storage and production conditions (6.7% and 8.1%), usually directly at the sales site. Although some people actually visit the kitchens of producers to check on the cleanliness of the production area. If the munkoyo expires, not everyone (28,4%) throws it away. Expiry is perceived different by each person. It is described as munkoyo being either too bitter (16.4%), too sour (19.4%) or too alcoholic (20.1%). Various periods of time are assigned to the expiring of munkoyo/chibwantu, ranging from a few days to a few months if kept refrigerated. Perceived risks associated with munkoyo mostly have to do with the hygiene (11.2 for tools, 9.4% for fermentation), wrong packaging (8.2%) and the use of poisonous munkoyo roots (9.4%).

One would expect that commercial processes are considered safer, however, only 40% (against 30.8%) expected commercial mahewu to be safer than traditional munkoyo. The most mentioned reason why commercial is safer was that the production is controlled in various ways (50% of people that answered commercial products are safer). The most mentioned reason why traditional would be safer is that the people know what goes inside since they know the traditional process (54% of people that answered traditional). 60% of the interviewed Zambians contribute to the safety (and shelf life) themselves by always keeping their munkoyo refrigerated.

78.9% of the interviewed people never had any symptoms of illness after drinking munkoyo. The remaining people (10.8%) mostly experienced discomfort only once. However there is little personal experience, 40% did (indirectly) know someone that did get symptoms after drinking munkoyo. For both the own and other's experience, diarrhoea and abdominal cramps were the most frequently suffered symptoms. For other's experiences, vomiting was almost as frequently mentioned as diarrhoea. Further mentioned symptoms were nausea, headache, dizziness, muscle weakness, blurred/double vision and even death. Most *combinations* of symptoms found can be related to food borne infection (food containing bacteria that grow in the intestinal tract) or food intoxication (food containing toxins produced by bacteria in the food). The people that got ill themselves got abdominal cramps (27.8%) or abdominal cramps and diarrhoea (27.8%) most of the times. 22.2% just got diarrhoea. For experiences of friends the most mention symptom was just diarrhoea (30.4%), following by the combination of abdominal cramps and diarrhoea (21.7%) or diarrhoea and vomiting

(17.4%). The most mentioned reason for illness was over-fermentation (23.4%), followed by various other reasons (22.1%) unhygienic cooking tools (14.3%), wrong packaging and unhygienic fermentation (both 11.7%) and wrong storage of the finished product (9.1%). The exact definition of over-fermentation differed per interviewee, but was described as munkoyo/chibwantu that is bitter, too sour, alcoholic or bubbly.

Group-specific answers

There were no relationship ($p > 0.05$) between demographic factors (education, age, residance) and knowing what food poisoning is or perceiving munkoyo/chibwantu as safe or not. Higher educated people associate commercial mahewu with a higher level of safety ($p < 0.05$) than lower educated people. Residence or age do not influence this preference. Even though there is a difference in perception of safety, none of the demographic factors is related ($p > 0.05$) to the preference for commercial or traditional. The majority of the people prefers traditional munkoyo/chibwantu over commercial mahewu.

Quantitative producer questionnaire

General and process

The complete outcome of the questionnaire can be found in table 8 in the appendix. Boiling time varied per producer but comprised 1-2 hours for 43,8% of the producers. By rounding up the boiling time (e.g. <1 h becomes 60 minutes), an estimation of the average boiling time per product was calculated. The longest boiling time was found for munkoyo, followed by burnt munkoyo and the least for chibwantu, but there was no significant difference ($p > 0.05$). The average temperature at which the root (extract) is added is highest for chibwantu. However, if the outlier of 100 ˚C (Ci4) is taken out the average temperature at which the root is added becomes better comparable. Both chibwantu and burnt munkoyo are cooled down till 42 ˚C, munkoyo is cooled down till 33 ˚C. 56,3% do not cover the munkoyo during cool down, which could cause infections from the environment. However, it also causes the munkoyo to cool down faster, reducing the time in which pathogens could start growing. One producer argued that the steam coming off the munkoyo scares away flies. Burnt munkoyo is always sieved to remove the roots and burnt particles. Chibwantu is not always sieved, since the product should have chunks inside. Normal munkoyo is not always sieved. Most interviewed producers sell their product at the local market (42,1%) or from their home or restaurant (26,3%). Fermentation time varied from 8 (overnight) to 24 hours (1 day). The average fermentation time was 12,92 ± 7,69 hours for all products.

Munkoyo root and raw materials

All producers think that the munkoyo root can pose a risk, but when asked to describe the wrong root, several producers admit not to know what it looks like. The ones that do have an idea, mention that the smell and taste is different: bitter. The real root is sweet. Furthermore the leaf and size are an indicator. Four of the producers also argue that the white or opposite the yellow one is poisonous, which was also seen in the research of Mwale (2014). This conflicts with the choice of some producers to work with the white whereas other rather work with the yellow root. One producer also mentions that the colour becomes lighter as the roots are more dry, which could confuse producers when picking the right root. 6 out of 15 producers that were questioned, use whole munkoyo roots. The other 9 make an extract using water.

The longer munkoyo stands, the more alcoholic it gets. To be able to sell it as munkoyo, some producers choose to stop fermentation. They do this by cooling the product (38,9%) or by transferring it to another container (27,8%), which does not really stop fermentation, but can slow down the process since the product is no longer in contact with the calabash that potentially contains a biofilm that could speed up fermentation.

10 out of 13 producers use cold, unboiled water to clean their tools. 9 out of 10 use soap. 1 uses water that is first boiled. The remaining 2 did not specify what kind of water they use for cleaning. Even if the soap eliminates bacteria, it is wise not to just rely on the soap to kill bacteria, but rather treat the water or use water from a reliable source that is regularly checked on presence of pathogens. Disinfectants can also get rid of bacteria, but the producers mentioned that they do not want to use soap or disinfectants in the fermentation containers, since that changes the flavour of their munkoyo. If these containers indeed contain biofilms, that could be the reason for the alteration of the munkoyo after disinfection.

2 producers measured the time of the process, 1 used a scale to weigh ingredients and 9 used cups to determine the amount of ingredients. This indicates the low level of standardization that is used for the production of munkoyo. The producers that were interviewed varied from home-scale to small enterprises. Time-measuring was only performed by the two biggest producers. This indicates that munkoyo production throughout the country at all levels still can be greatly improved when it comes to standardization.

Cleaning of the munkoyo root was either not done (38,5%) or done by hand (53,8%). Furthermore half of the producers used water, the other half did not. Water mostly came from a pipeline (61,5%) or a borehole (30,8%). Only 4 out of 13 boiled the water before use. This step can introduce many pathogens into the munkoyo. The high incidence of manual handling can cause *S. aureus* to invade the process, but also the use of unboiled water forms a major risk if the water source is contaminated. When asked if the munkoyo is diluted, most producers answer that the munkoyo root extract functions as diluter. Not only because of the extra liquid, but also because of breakdown of the starches in the porridge by amylases from the munkoyo root. The addition of the munkoyo root extract is always done after cooking. One producer mentions that if you want to add water, you should do it during the boiling of the porridge, since it will not mix properly with the porridge if added afterwards. A bit more than half of the producers (53,8%) adds sugar to the munkoyo. This makes the product tasty, but can also provide substrate for possible pathogens.

Everyone stores their raw materials at room temperature in closable bags, buckets or bins. 53,3% of the producers uses repellent to keep away insects. 20% uses a cat to scare away rodents or geckos. The perceived characteristics of when munkoyo is considered to be spoiled vary greatly, but 8 out of 13 producers think munkoyo is spoiled the moment it becomes alcoholic. 5 out of 13 further comment that sour- or bitterness are indicators of spoilage. Therefore 6 out of 13 producers discard their product if it becomes sour or alcoholic. 5 out of 13 keep on selling the product or drink it themselves.

The most mentioned measures taken to ensure hygiene are washing of the surroundings and tools (12 out of 13) and hands (11 out of 13), checking of the raw material and wearing hairnets or equivalents (10 out of 13). Furthermore 5 out of 13 took off their jewellery and 4 out of 13 cool their product and check it visually before selling.

Qualitative interview

Quality perception

Before any recommendations on the production process can be made, it is important to determine what local producers perceive as quality; a definition of what their product should be like or conform to. All producers agreed that qualitative product's properties should be consistent, so that customers know what they will get. It is important to use high quality ingredients (no moulds, good taste et cetera), and keep it "natural", so no additions of chemicals or preservatives, which could be called the Zambian "*Reinheitsgebot*" for Munkoyo. The product should be thick. In the case of burnt munkoyo, the mixture should be brown, not black. Three out of four producers added that no one should ever get ill by consuming their product. One smaller producer stressed the importance of cooking on fire fueled by fire wood instead of coals or electricity to obtain the right, slightly smoky taste.

Ensuring quality

To guarantee quality, several measures are taken. First of all, all producers claim to use high quality ingredients that they pick out themselves or by someone they personally appointed. They check the raw materials visually and sieve out foreign objects in the case of munkoyo. Chibwantu is not sieved, but washed. The grits float and stones or other objects sink.

Second, the two bigger producers (>50 liters per day) have a manager that continuously checks on the employees to ensure they work hygienically. Most workers are illiterate and thus trained orally. If they do not comply with the rules, they are fired. The workers have to wash themselves before starting production. If someone does not comply with the rules, the person is fired. One big producer mixes batches made by different employees to get a more constant result, which can be considered as a simple method to ensure product quality.

Third, the bigger producers use transparent bottles to show that their product looks as it should. One of the big companies also washed the bottles before filling them to remove possible contaminant from the bottle. Both companies wash the bottles after filling so that the outside of the bottle will not become moldy or sticky.

Fourth, all producers claim to perform good hygiene practices. Three out of four producers demonstrated their process. Indeed, they thought their processes through and did whatever was necessary to ensure a safe product within their knowledge. However, the circumstances still would not comply with the Codex Alimentarius for the Street-Vended Foods in Africa set by the FAO (Joint FAO/WHO, 2001). The taken measures were based on what the producers knew by experience or common sense, not on knowledge obtained from official guidelines or institutions. A first example is that the shown processes all took place mostly outside, making it easy for air-borne contaminants to get into the product. Two out of three producers that showed their process did not cover the containers during cool down or the buckets in which the munkoyo roots were soaked in water. The buckets used were mostly standing directly on the ground or floor. Everyone made sure that foreign objects were removed, either by sieving or checking manually in the case of chibwantu production. No one performed monitoring measures like measuring the temperature or pH at critical moments. Also there are no tests available to check if a non-poisonous munkoyo root is used and there is no way to control possible contaminations coming from the roots, since they are added after boiling. Cooling down of the porridge was not controlled and done by leaving the pot outside, making it

possible for potential *B. cereus* spores to start growing in the porridge. No one ever had their samples check on microbial contaminations. One producer adds the munkoyo root extract right after boiling to kill potential pathogens. Another producer temporarily stopped producing due to a cholera outbreak out of fear that people would link possible cholera infections to her product. Both measures show certain awareness about pathogens, but they also indicate that producers lack detailed knowledge on how to prevent food borne diseases. Since these producers represent producers that already have an upscaled production, it could be suggested that none of the producer of munkoyo/chibwantu perform such measures throughout the country. Even though munkoyo is safe to consume most of the times, there are still annual cases of food poisoning or hospitalization after consumption of the product, making it important to inform producers on were the process's risks are and how to minimize them.

Some measures that were observed did comply with the FAO's guidelines though. The two bigger producers boil all water prior to use. Sick people are not allowed to cook the product. One producer lets the employees get a medical check-up yearly.

Improvements

The producers were asked what they would improve to produce even better and safer products. The two big producers would like to have automated pots, fillers and measuring tools like a temperature and pH meter. They would both like to have a safety plan, but do not know how to make one themselves. Everyone agrees that, since most people are illiterate, it is also important to describe instructions using cartoons. One big producer mentioned that he thinks that air-conditioning would be an improvement. The smaller companies would like to have their own bottles and labels first, before they start thinking about other things. When asked about a safety plan, they have no clue what that would be. One mentions that her safety plan is just to produce a few times, let the people buy it and if they then trust you just keep on producing. 3 out of 4 also mention that you can keep safety under control by making small batches that sell quickly. 1 big one makes enough so it sells within a week. Both big ones want to reduce manual handling. They are a bit afraid that pipelines and big pots might be hard to clean and that you have to use chemicals in that case. It is very important for all producers not to use additives or chemicals. It should be natural. 1 big one would like to have a manufacturing license. Trust is a word often heard, more important than certificates. It is obtained via mouth to mouth advertisement. Also movies of factory environments on social media could help. This is in line with the consumer questionnaire, in which it was found that 71,8% of the people prefers to get their munkoyo from a familiar sourced. The suggested improvements could be used to determine which steps should be undertaken first to upscale the production of munkoyo/chibwantu in such a way that the approached producer agrees and is willing to collaborate.

Conclusion

Customers

The majority thinks it is safe or remotely safe (97,2%) and prefers traditional over commercial munkoyo (84.2%). Trust is important when buying munkoyo. Since knowing each customer is not achievable, trust should be gained in another matter. For example by filming in the factory and using social media to communicate with customers, making it able for them to directly ask questions and see the production process, so that they know what goes into the product. By filming, the customers can also see that the hygiene is well. It can also be used as a manner to describe which kind of munkoyo roots or alternative are used, since that is also seen as a risk. Furthermore see-through bottles should be used so that the customer can see what he/she is buying. Most consumers (78,9%) never experienced any symptoms of illness after drinking munkoyo. The remaining people mostly experienced abdominal cramps, diarrhea and vomiting

Producers

Several measures or actions were observed that contribute to the safety of the product. 4 out of 13 boil water before making munkoyo root extract. Everyone used closable bags and bins for the raw materials. 53.5% uses repellent against insects and rodents. 20% uses a cat, 6 out of 13 producers throw away their product if it becomes sour or alcoholic. 9 out of 10 use soap to clean. 12 out of 13 clean their surrounding and tools, 11 wash their hands and 10 wear hairnets or equivalents and thoroughly check the raw materials. 5 took of their jewellery and 4 cooled their end product and checked it visually once more.

However we observed several issues that could reduce the product's safety. No one had a method to speed up cooling down of the porridge and 56.3% did not cover the container with boiled porridge, which gives pathogens or spores the chance to enter and grow. There is no method yet to make sure that the right munkoyo root is used. Also, the munkoyo root is touched by many people before adding it to the porridge. After fermentation munkoyo is usually sieved, but chibwantu is not. Any foreign objects could still be present in the drink. The end of fermentation (the product is ready for consumption) is further determined visually or by taste. To ensure a safe product on an industrial scale, the pH should be measured. In the cold season fermentation can be slower, which gives pathogens the chance to grow for longer. When produced on a bigger scale, the temperature of the fermentation vessel should be controlled. Also, sugar that is sometimes added is best cooked with water first, so that potentially present pathogens are inactivated. 5 out of 13 did not throw away their product when it became spoiled according to their definitions. A shelf life of the product should be determined. Most producers did not cool their final product. Furthermore everyone had manual contact with the product after boiling and fermentation via addition of the munkoyo roots or selling the product. Last, 10 out of 13 producers did not boil or treat the water they use for cleaning, posing another possible introduction of pathogens into the production line.

Recommendations for safe production

This section contains a HACCP-plan for household and small scale production of munkoyo/chibwantu based on similar work done by Motarjemi (2002). The steps that are not applicable in households indicated in *italic.* The HACCP-plan is followed by general recommendations for both small and large-scale producers. At last, a cartoon is added so that also illiterate people can be informed with fundamental steps of safe food production.

Step	Hazards	Control measures	CCPs	Critical limit	Monitoring procedure	Corrective action
1. Raw materials	**a.**					
a. Maize	i. Mycotoxins	i.1. Limit storage time and store the maize in a dry and (wherever possible) cool area 2. Get assurance/information on proper handling conditions by the supplier	i. Yes	i.1. No mould, right smell 2. Humidity, time and temperature in storage area	i.1. Visual control, smell. Monitor temperature, time and humidity if possible 2. Obtain information on storage conditions and time supplier	i.1. Discard maize, change supplier in case product arrived moldy 2. Change supplier in case storage/handling of supplier is not proper
	ii. Agrochemicals	ii. Get assurance/information on proper handling by the supplier/farmer	ii. No			
	iii. Pathogens (from soil and handling)	iii.1. Heat treatment 2. Quick cool down 3. Fast fermentation	iii. No			
	iv. Foreign objects (insects, stones etc.)	iv. Visual checking/manual cleaning (with water)	iv. Yes	iv. No visible foreign objects	iv. Sieving if possible, visual check and manual cleaning	iv. Re-sieve/clean
	b.					
b. Munkoyo root	i. Mycotoxins	i.1. Limit storage time and store the maize in a dry and (wherever possible) cool area 2. Get assurance/information on proper handling conditions by the supplier	i. Yes	i.1. No mould, right smell 2. Humidity, time and temperature in storage area	i.1. Visual control, smell. Monitor temperature, time and humidity if possible 2. Obtain information on storage conditions and time supplier	i.1. Discard maize, change supplier in case product arrived moldy 2. Change supplier in case storage/handling of supplier is not proper

	ii. Pathogens (from soil and handling)	ii. 1. Heat treatment 2. Quick cool down 3. Fast fermentation	ii. No			
	iii. Foreign objects (insects, stones etc.)	iii. Visual checking/manual cleaning (with water)	iii. Yes	iii. No visible foreign objects	iii. Sieving if possible, visual check and manual cleaning	iii. Re-sieve/clean
c. Crystalline Sugar	**c.**					
	i. Pathogens (in case production area is not hygienic)	i. Get assurance/information on proper handling conditions by the supplier	i. No		i. Optional: first boil sugar in water for 1 minute to eliminate pathogens	
	ii. Foreign objects (insects, stones, dust etc.)	ii. Visual checking/manual cleaning	ii. Yes	ii. No visible foreign objects	ii. Sieving if possible, visual check and manual cleaning	ii. Re-sieve/clean
d. Water	**d.**					
	i. Pathogens	i. Use boiled water in case no safe (treated) water is available	i. Yes for step 3, no for step 4.	i. For step 3: boil water beforehand for 1 minute, even if the water source is known		
	ii. Chemical contamination	ii. Get assurance/information of the supplier	ii. Yes	ii. Clear and free of off-flavors or taste	ii. Smelling, visual control, tasting	ii. Change water supplier
2. Grinding of maize	i. Introduction of pathogens via water in case of wet-milling	i. Boil porridge properly in boiling step	i. No			
	ii. Introduction of foreign objects	ii. Sieve the mealie meal or check visually	ii. Yes	ii. No visible foreign objects	ii. Sieving if possible, visual check and manual cleaning	ii. Re-sieve/clean
3. Soaking of root	i. Introduction of pathogens from handling	i. Wash hands properly with soap and a nail brush or wear gloves	i. Yes	i. No dirt under nails or on hands	i. Wash hands for at least 30 seconds and use soap	i. Rewash hands
			ii. Yes		ii. Measure boiling time	ii. Reboil water

	ii. introduction of pathogens via water and munkoyo root (see step 1)	ii. See step 1 “water” and “munkoyo root)		ii. Boil water for 1 minute, cool down		
	iii. Introduction of foreign objects	iii. Keep out flies or other foreign objects	iii. Yes	iii. Cover soaking vessel	iii. Keep the vessel covered	iii. Discard munkoyo water or boil it and soak new munkoyo in it
4. Boiling porridge	i. Survival of pathogens and spores	i. Boil the porridge properly to kill livings cells and inactivate most spores	i. Yes	i. Boiling for at least 90 minutes (Coleman et al., 2010)	i. Measure boiling time	i. Reboil for the time remaining. *If the temperature has dropped under 60 °C, reboil for 90 minutes*
5. Cool down	i. Germination of spores	i. Cool down the porridge as quickly as possible	i. Yes	i. Cool down to room temperature within 4 hours	i.. Feel the temperature of the porridge and measure time. *Measure the temperature of the porridge.*	i. Reboil the porridge for a few minutes. Discard the porridge if it has been standing around at body temperature *(30-40 °C*) for more than 4 hours
	ii. Introduction of pathogens via flies	ii. See 3.iii	ii. Yes	ii. No flies in the porridge	ii. Cover cooling vessel with a net that lets out heat, but leaves out insects	ii. Remove the fly and reboil the porridge for a few minutes
6. Fermentation	i. Outgrowth of *S. aureus* and other pathogens introduced through manual handling/munkoyo root	i. Rapid fermentation	i. Yes	i. Acid taste and characteristic odor within 24 h	i. Visual control, taste. *Measure pH, should be <4.0.*	i. Discard the product
	ii. Outgrowth of moulds	ii. Removal of moulds from top layer	ii. Yes	ii. No visible moulds	ii. Visual observation	ii. Remove more of the top layer or discard the whole product if very moldy

	iii. Introduction of pathogens via flies	iii. Keep out flies or other foreign objects	iii. Yes	iii. Cover fermentation vessel	iii. Keep the vessel covered	iii. Scoop out the fly and surrounding fluid carefully or discard the munkoyo if a lot have come in or the fly has been in the munkoyo for long
7. Sieving of product	i. Recontamination by tools/hands	i. Wash hands properly and use clean tools	i. Yes	i. Wash hands as described in step 3, rinse tools and use detergent if possible	i. No dirt on hands/nails/tools	i. Rewash
8. Sweetening	i. No hazard (see "sugar")					
9. Filling of bottles	i. Recontamination by tools/hands	i. Wash hands properly and use clean tools	i. Yes	i. Wash hands as described in step 3, rinse tools and use detergent if possible	i. No dirt on hands/nails/tools	i. Rewash

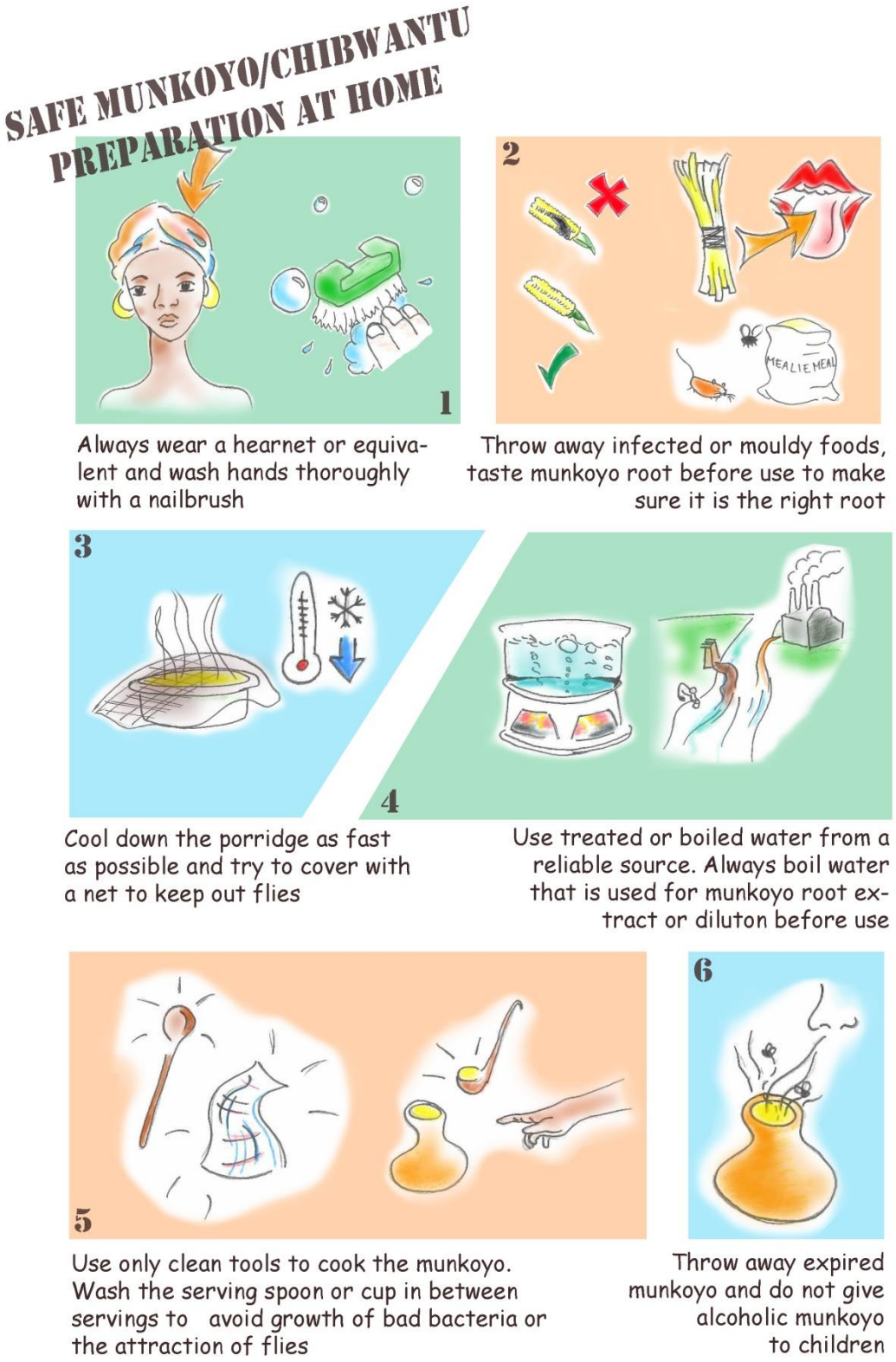
Home scale and larger scale

- Ensure good personal hygiene by washing hands with warm water and soap before handling the product or after visiting the toilet. Use a brush to clean underneath the nails.
- Wear clean clothes and a hair net or equivalent during production. Change towels regularly.
- Keep the surroundings where the raw materials and finished product are stored clean and free of rodents and insects. Try to keep the humidity and temperature in storage areas as low as possible.
- Wash the tools used for production thoroughly with treated or boiled water. Use detergent if available, but not for the fermentation vessel.
- Boil all water prior to use that is needed for munkoyo root extract or dilution.
- Taste the munkoyo root before you add it to confirm that it is the same root you always use.
- Cover the cool down vessel or product at all times to avoid entry of flies or other foreign objects.

Large scale only

- Try to cool down the porridge as fast as possible. Use a thermometer to time the moment when to add the munkoyo root.
- Use a pH-meter to make sure the final product has a pH below 4.
- If sugar is added, first dissolve it in water and boil it for a few minutes to kill any microorganisms present in the sugar.
- Disinfect the funnels/bottles/tools used to touch the product after fermentation is done to avoid contamination of the end product. Do not touch the finished product with hands. Gloves can be used, but care must be taken that nothing but the product or the tools directly needed to further process the product are touched.
- Educate employees about hygiene and food safety orally and written. Use cartoons to explain and to remind your employees in case they are illiterate.
- Make sure that the source of the munkoyo root is known and consistent wherever possible. Wash the roots properly before use by using boiled water.
- Always sieve the final product to remove any foreign objects. Use a coarser sieve for chibwantu.

Safety comic



Recommendations for future research

For this thesis only a small amount of plants from two locations were sampled. However, the plants used for munkoyo differ throughout the country. This also implies that the wrong plants that are sometimes used for munkoyo are also different. It would be useful to visit multiple areas throughout Zambia, take samples of both the right and wrong roots and determine their species. If it is indeed the case that some roots are poisonous, these poisons can perhaps be extracted and determined. Based on this information, possibly tests can be developed to help munkoyo producers in picking the right root.

Since in this thesis it did not turn out that the fermentation of munkoyo eliminates pathogens, preservation techniques that kill pathogens should be considered when up scaling munkoyo production. A cost and feasibility analysis for different preservation techniques should be made. Furthermore the influence of preservation on the aroma profile and organoleptic properties of munkoyo should be added.

A difference was found in the ethanol content for the various samples, the reason for this should be explored further, so that an alcohol-free/low product can be produced. For example: the effect of calabashes or buckets on the ethanol content. This can be useful to pick fermentation vessels when producing on larger scale.

Since the northern-type munkoyo is burned deliberately, it should be measured how many carcinogenic compounds the munkoyo contains. Using a sensorial study, the minimum for flavor acceptance can be determined. This way the amount of burning can be kept at a minimum and therefore the risk of adverse health effects can be lowered.

Last it would be interesting to try out the invasion experiment with burnt munkoyo and munkoyo with sugar added right before fermentation. Also various fermentation temperatures and their influence on the resilience of pathogens, microbial community and aroma profile could be tried out. Perhaps these factors influence the fermentation in such a way that pathogens die of faster.

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Appendix

Appendix A

Pathogen detection

		Pathogen				Group of microorganisms				
Sample	pH	*S. aureus*	*B. cereus*	*Shigella*	*Salmonella spp.*	Enterobacteriaceae	TVC	Lactobacilli	Lactic streptococci	Yeasts and moulds
Bm 1	3,52 ± 0,00	2,8	2,3	-	-	2,8	7,5	5,5	5,1	5,7
Ci 1	3,71 ± 0,00	3,0	< 2,0	-	-	2,9	7,4	7,9	4,7	2,7
Ci 4	3,51 ± 0,00	< 2,0	< 2,0	-	-	1,7		7,9	6,5	< 3,0
Ci 5	3,31 ± 0,00	< 2,0	< 2,0	-	-	2,1		7,8	6,6	6,4
Bm 4	3,28 ± 0,00	< 2,0	3,0	-	-	4,0		8,4	8,1	5,7
Bm 5	3,90 ± 0,00	3,6	< 2,0	-	-	3,5		8,6	7,3	5,2
Ci 6	3,76 ± 0,00	< 2,0	< 2,0	-	-	2,3	8,1	7,9	7,5	6,0
Ci 7	3,81 ± 0,00	< 2,0	< 2,0	-	-	1,8	7,8	8,1	8,5	5,8
Mu 1	4,50 ± 0,01	< 2,0	5,7	-	-	4,7	6,8	6,7	5,5	4,9
Mu 2	3,88 ± 0,02	4,5	< 2,0	-	-	< 1,0		7,5	6,7	4,8
Mu 3	3,71 ± 0,00	5,4	< 2,0	-	-	4,4	> 9,5	> 8,6	8,4	5,4
Bm 2	3,06 ± 0,01	< 2,0	2,7	-	-	1,0	6,1	6,7	4,8	4,8
Bm 3	3,14 ± 0,00	< 2,0	4,3	-	-	4,4		8,4	8,1	6,3
Ci 2	3,47 ± 0,03	4,2	< 2,0	-	-	4,9	7,7	7,5	5,5	4,6
Mu 4	3,54 ± 0,00	< 2,0	< 2,0	-	-	3,3		8,5	6,0	5,7
Ci 3	5,14 ± 0,04	4,7	7,3	-	-	1,7	7,7	8,3	> 6,5	3,1
	Amount tested positive	7	6	0	0	15				

Table 4 Presence and numbers of pathogens and microorganisms in munkoyo. Bm = burnt munkoyo, ci = chibwantu and mu = munkoyo

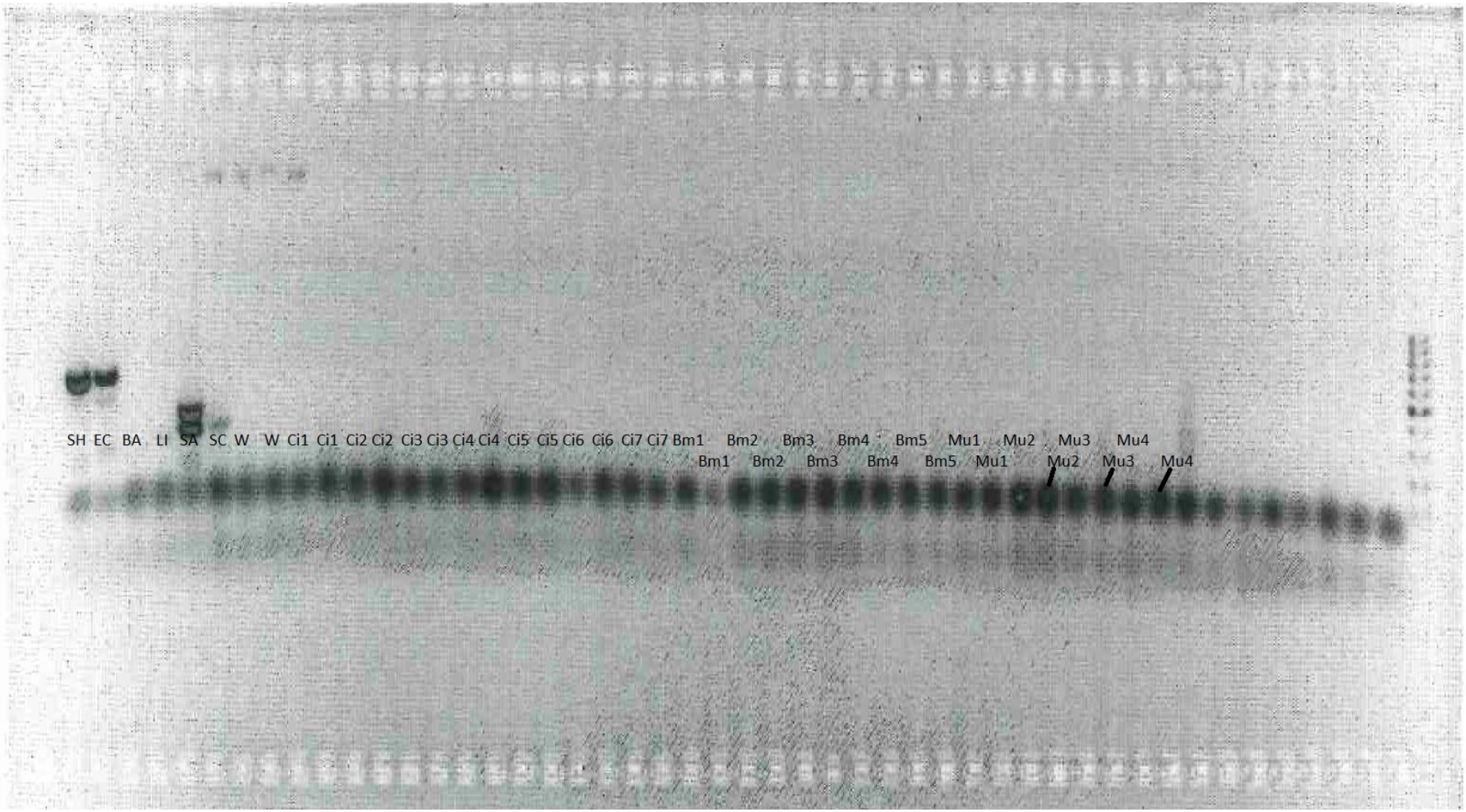

Figure 6 PCR-reaction products on agarose gel of all munkoyo/chibwantu samples taken from Zambia (Ci = chibwantu, Bm = burnt munkoyo, mu = munkoyo) and the positive controls (SH = *shigella,* EC = *E. coli*, BA = *B. cereus*, SA = *Salmonella,* SC = *S. aureus*)

Test	Compared between	What	Significance	Significant?	Degrees of freedom	Comments
ANOVA	Product groups	Enterobacteriaceae	0.545	No	2	
		Lactic Streptococci	0.997	No		
		LAB	0.730	No		
		Yeast and moulds	0.316	No		
	Root extract user	Enterobacteriaceae	0.883	No	1	
	Age groups product	pH	0.035	Yes	3	Not finished fermenting left out
	Product groups		0.608	No	2	
Fisher's exact test	Sieving of product	Presence of *S. aureus*	0.284	No	1	
	Age groups product		0.890	No	3	Not finished fermenting left out
	Fermentation time	Presence of *B. cereus*	0.305	No	1	
	Age groups product		0.556	No	3	Not finished fermenting left out

Table 5 Statistical tests and their significance for chapter 1

Appendix B

Significance tests product

Test	Compared between/within	What	Significance	Significant?	Degrees of freedom	Comments
ANOVA	Product groups	Ethanol content	0.000	Yes	2	If outlier is excluded
	Fermentation container		0.389	No	3	
	Boiling time		0.306	No	3	
	Root extract user		0.714	No	1	
	Addition of root moment		0.855	No	5	
	Age groups product		0.607	No	3	
	Product groups	Citric acid	0.357	No	2	
		Formic acid	0.402	No		
		Acetic acid	0.037	Yes		
		Lactic acid	0.311	No		
	Fermentation container	Citric acid	0.426	No	3	
		Formic acid	0.730	No		
		Acetic acid	0.494	No		
		Lactic acid	0.284	No		
	Boiling time	Citric acid	0.397	No	3	
		Formic acid	0.194	No		
		Acetic acid	0.347	No		
		Lactic acid	0.319	No		
	Addition of root moment	Citric acid	0.770	No	5	
		Formic acid	0.001	Yes		Only 2 samples contain formic acid
		Acetic acid	0.406	No		
		Lactic acid	0.259	No		
	Age groups product	Citric acid	0.426	No	3	
		Formic acid	0.528	No		
		Acetic acid	0.310	No		
		Lactic acid	0.078	No		
One-sample Test	Central/eastern-type	Ethanol content	0.985	No	3	
	Chibwantu		0.919	No	6	If outlier is excluded
	Northern-type		0.994	No	4	
	Central/eastern-type	Lactic acid	0.955	No	3	
	Chibwantu		0.015	Yes	6	
	Northern-type		0.975	No	4	

	Central/eastern-type	Acetic acid	0.745	No	3	
	Chibwantu		0.034	Yes	6	
	Northern-type		0.868	No	4	
Pearson correlation	Yeast and moulds	Ethanol content	0.037	Yes	15	
	Enterobacteriaceae	Citric acid	0.138	No		
		Formic acid	0.387	No		
		Acetic acid	0.050	Yes		
		Lactic acid	0.986	No		
	Lactic streptococci	Citric acid	0.143	No		
		Formic acid	0.880	No		
		Acetic acid	0.350	No		
		Lactic acid	0.464	No		
	LAB	Citric acid	0.208	No		
		Formic acid	0.325	No		
		Acetic acid	0.908	No		
		Lactic acid	0.309	No		
	Yeast and moulds	Citric acid	0.861	No		
		Formic acid	0.821	No		
		Acetic acid	0.196	No		
		Lactic acid	0.105	No		

Table 6 Statistical tests and their significance chapter 2

Appendix C

Traditional fermented munkoyo/chibwantu consumer questionnaire

[University of Zambia UNZA, Wageningen University WUR]
Ivana Mik

Please fill in the blanks and place an X next to the word or phrase that best matches your response. For responses with the answer [other] please fill in the answer on the dotted line.

Date: ……..

Name enumerator:……..

What is your gender?

- [ ] Male
- [ ] Female

What is your age?

- [ ] Under 20
- [ ] 20 – 29
- [ ] 30 – 39
- [ ] 40 – 49
- [ ] 50 – 65
- [ ] Over 65

What is your occupation?

- [ ] Student
- [ ] Unemployed
- [ ] (Self-)Employed
- [ ] Retired
- [ ] Other:……..
- [ ] Prefer not to say

What is your highest level of education?

- [ ] No education
- [ ] Primary (Grade 7)
- [ ] Secondary (O-Level or A-Level)
- [ ] Vocational training
- [ ] College diploma
- [ ] Bachelor's Degree
- [ ] Master's Degree
- [ ] Doctorate Degree

Tribe: ……

Current residence: ……..

Please circle the word that best matches your response. Note that commercial products comprise products like mahewu.

1. Which product do you consume: Munkoyo / Chibwantu / Both
2. How often do you consume munkoyo/chibwantu: Daily / Weekly / Monthly / Yearly
3. What production method do you prefer: Traditional / Commercial / No preference
4. Where do you get your traditional munkoyo/chibwantu: I make it myself / Local market / Mini-mart / Filling station / Supermarket / From friends or family / Other, namely……
5. Do you think it is safe to consume traditional munkoyo/chibwantu? Yes / No / Do not know
   If no was answered, why?

	I associate no risks with traditional munkoyo/chibwantu		Unhygienic packaging of product
	Unhygienic tools for cooking process could be used		Use of dirty/contaminated water for making munkoyo root extract
	Unhygienic tools for fermentation could be used		Use of dirty/contaminated water for diluting munkoyo
	Black magic or added poison		No preservatives
	Wrong munkoyo root could be used		Wrong storage of the finished product
	Wrong storage of the raw materials		Other, namely ……

6. How do you make sure for yourself that the traditional munkoyo/chibwantu that you drink is safe?

	Get it from familiar source/supplier		Visual inspection of product (color/viscosity/other)
	Produce it myself		Smelling of product
	Visual inspection of storage conditions of trader		Asking producer questions on production of product
	Visual inspection of production area of trader/producer		Other, namely…

7. Which one do you think is safer to consume: Traditional / Commercial / Both as safe
   If a preference is given, why? .....
8. Do you drink the bought traditional munkoyo/chibwantu immediately: Yes / No
9. If no, how do you store your munkoyo/chibwantu: ……
10. When do you stop drinking munkoyo/chibwantu: Too bitter / Too acidic / Too alcoholic / Slimy / Never discard / Other, namely……
11. Do you know what food poisoning is? Yes / No

12. Did you ever had any physical inconvenience after consumption of traditional munkoyo/chibwantu (place an X next all that apply):

	I have never had any problems		Blurred or double vision		Nausea		Paralysis
	Abdominal cramps		Fatigue		Headache		Increased gas
	Diarrhea		Muscle ache		Dizziness		Slurred speech
	Bloody diarrhea		Loss of appetite		Muscle weakness		Death
	Vomiting		Fever		Difficulties in breathing		Other, namely……

13. If you experienced any discomfort, how often do you experience this after consumption of munkoyo/chibwantu:
    Always / Frequently / Sometimes / Once / Other, namely……
14. Do you know someone else who got ill from drinking traditional munkoyo/chibwantu: Yes / No
15. If you do know someone that got ill, do you know which symptoms the person suffered?

	I do not know which symptoms the person suffered		Blurred or double vision		Nausea		Paralysis
	Abdominal cramps		Fatigue		Headache		Increased gas
	Diarrhea		Muscle ache		Dizziness		Slurred speech
	Bloody diarrhea		Loss of appetite		Muscle weakness		Death
	Vomiting		Fever		Difficulties in breathing		Other, namely……

16. If you or anyone else you know has experienced discomfort after consuming traditional munkoyo/chibwantu, what do you think was the reason for this?

	Wrong munkoyo root		Unhygienic tools for storage of product
	Overfermented		Unhygienic packaging of product
	Unhygienic tools for cooking process		Use of dirty/contaminated water for making munkoyo root extract
	Unhygienic tools for fermentation		Use of dirty/contaminated water for diluting munkoyo
	Black magic or added poison		Other, namely ……

Quantitative survey munkoyo/chibwantu producers

This survey should be combined with 1 end product per producer, which is tested for presence of pathogens.

Producer name and/or location: …….
Date of interview: ……./……../……..

Sales

1. What product(s) do you produce (tick all that apply)?

- Chibwantu
- Munkoyo
- Burnt munkoyo
- Other, namely ……

2. For who do you produce your product (tick all that apply)?

- Own use
- Friends and family
- For commercial use

If 2. was answered with "for commercial use", answer question 3:

3. Where do you sell your product (tick all that apply)?

- At local markets
- At bus stops
- In mini-marts
- In supermarkets
- Other, namely ……

4. How often do you produce your product?

- Daily
- A few times per week
- Once per week
- Monthly

5. How much of your product do you produce each time?

- <5 liters
- 5-15 liters
- 15-30 liters
- 30-50 liters
- >50 liters

6. How do you sell your product?

- Directly from the fermentation vessel in containers brought by customers
- In prepackaged bottles/containers
- Other, namely ……

Process

7. Can you fill in the following production parameters for you process?

Parameter	
Burning/overcooking) of the porridge	Yes/no
Approximate boiling/cooking time) time of porridge	o <1 hour o 1-2 hours o 2-3 hours o >3 hours
Cooling down of porridge before adding munkoyo root (extract)	o No cooling down o Slightly painful when touched o Luke warm o Body temperature o Room temperature o Below room temperature
Is the container in which the product cools down covered with something?	Yes/no If yes, with:……
Fermentation time	……hours/days
Fermentation vessel	o Plastic bucket o Metal container o Calabash o Other, namely……
Is the fermentation vessel covered with something? If yes, with what?	Yes/no If yes, with:……
Sieving of end product	Yes/no

Further comments to question 7: ……
……
……

8. How do you know that the fermentation is done?

- o By tasting
- o Visually
- o By performing measurements with specific tools
- o Other, namely ……

Explain what you taste/see/measure:
……
……

9. How do you stop fermentation (according to producer)?

- o By transferring it to another container
- o By putting in the refrigerator/cooling it
- o By boiling the finished product
- o I do not stop the fermentation
- o Other, namely ……

10. Do you add sugar to the munkoyo/chibwantu? If yes, when?

- o I do not add sugar to the product
- o After fermentation
- o Other, namely ……

11. Did you standardize any of the following parameters of the process of making your product (tick all that apply)?

- o No
- o Measuring time
- o Measuring temperature
- o Measuring acidity
- o Measuring ingredients using cups/buckets
- o Weighing ingredients using a scale
- o Other, namely ……

Ingredients

General and storage

12. What ingredients do you use for your product?

- o Maize meal
- o Maize grits
- o Munkoyo root (whole)
- o Munkoyo root extract
- o Flour
- o Sugar
- o Other, namely……

13. Where do you store your ingredients and at what temperature?

Storage location: ……

Temperature: o Refrigerated/cooled o Below room temperature
o Room temperature o Above room temperature

14. Do you use anything to scare away insects/rodents (tick all that apply)?

Methods: o I do not use anything o Traps
o Cat o Repellant
o Other, namely ……

15. Do you ever discard ingredients if there are any defects? If yes, what defects?

Discarding: yes/no

Defects: o I never discard anything o Smelly water
o Mould on munkoyo roots o Insects in raw materials
o Rodent feces in raw materials o Odd looking maize kernels
o Mould on maize o Other, namely ……

Additional comments to question 15, in case further explanation is given:

……
……

Munkoyo root

16. What type of root do you use?

- o Yellow, dried
- o Yellow, fresh
- o White, dried
- o White, fresh
- o Other, namely…

16. Where do you get your roots?

- o Market
- o Via friends/family
- o Pick them yourself
- o Other, namely ……

17. How do you know which root to pick (tick all that apply)??

- o Color
- o Shape
- o Smell
- o Taste
- o Other, namely ……

18. Do you think some roots can be dangerous? If yes, what symptoms can they cause (tick all that apply)?

Dangerous: yes/no

Symptoms:

- o Abdominal cramps
- o Diarrhea
- o Bloody diarrhea
- o Vomiting
- o Increased gas
- o Fatigue
- o Muscle ache
- o Loss of appetite
- o Fever
- o Nausea
- o Headache
- o Dizzyness
- o Muscle weakness
- o Difficulties in breathing
- o Paralysis
- o Blurred or double vision
- o Slurred speech
- o Death
- o Other, namely…….

Comments to question 18, in case "death" was the answer: ……
……

19. If you answered question 18. with yes, can you describe what the wrong roots look like?

……
……

In case question 12. was answered with "Munkoyo root extracted with water", answer question 20:

20. How do you make the munkoyo root extract?

Parameter	
Root cleaning by hand	Yes/no
Root cleaning using water	Yes/no
Water source for extraction	o Borehole o Pipeline o Bottled water o Other, namely…
Boiling of water before use	Yes/no
In case of boiling, cooling down till	o Still very hot o Luke warm o Room temperature

Water

21. Do you ever dilute the porridge or finished product after the cooking process is complete? If yes, when do you add the water and is the added water boiled? At what temperature do you add it and what is the source of the water?

Dilution: yes/no
Addition: o Directly after cooking o During cooling down
o After cooling down o To the fermented product
In case the added water is boiled, it is cooled down till before addition:
o Still very hot o Luke warm o Room temperature

- o From a pipeline
- o From a well
- o Borehole
- o Other, namely ……

Hygiene

22. How do you clean your tools (tick all that apply)?

- o I do not clean my tools
- o Cold water
- o Boiled water which is cooled down
- o Hot water
- o Soap
- o Bleach/disinfectant
- o Other, namely ……

23. Do you ever discard the finished product? Why?

- o I never discard the finished product
- o Mould on product
- o Insects in product
- o Too acidic
- o Too alcoholic
- o Other, namely ……

24. What do you do to make sure the production of your production is done in a hygienic/safe way?

- o Wash hands
- o No jewelry
- o Hair nets (beard/head)
- o Wash all tools after use
- o Measure temperature at various points
- o Measure pH at various points
- o Measure time of process steps
- o Check raw materials visually
- o Inspect end product visually
- o Inspect end product's taste
- o Inspect end product's smell
- o Inspect end product's consistency
- o Make sure surroundings are clean
- o Cooling of finished product
- o Other, namely……

Consumption

24. Did you ever have any complaints/problems concerning your product? If yes, of what kind (Tick all that are applicable)?

- o I never had any complaints/problems from customers
- o Product is spoiled
- o Consumer got had discomforts after consumption
- o Consumer got seriously ill after consumption
- o Consumer died after consumption
- o Other, namely ……

If question 24. Was answered with discomforts/ill or death, fill in the symptoms that were suffered (tick all that apply):

Symptoms:

- o Abdominal cramps
- o Diarrhea
- o Bloody diarrhea
- o Vomiting
- o Increased gas
- o Fatigue
- o Muscle ache
- o Loss of appetite
- o Fever
- o Nausea
- o Headache
- o Dizziness
- o Muscle weakness
- o Difficulties in breathing
- o Paralysis
- o Blurred or double vision
- o Slurred speech
- o Death
- o Other, namely…….

In case question 24. was answered positively, answer question 25.

25. What do you believe was the source for the complaints/problems (Tick all that are applicable)?

- o Wrong munkoyo root
- o Wrong fermentation (if this answer is given, what went wrong according to the producer?
  ……
- o Unhygienic tools for cooking process
- o Unhygienic tools for fermentation
- o Unhygienic tools for storage of product
- o Unhygienic packaging of product
- o Use of dirty/contaminated water for making munkoyo root extract
- o Use of dirty/contaminated water for diluting munkoyo
- o Other, namely ……

26. Have you ever heard of any other producer that had problems with his/her chibwantu/munkoyo? If yes, of what kind (Tick all that are applicable)?

- o I never heard of any complaints/problems from other producers
- o Product is spoiled
- o Consumer got had discomforts after consumption
- o Consumer got seriously ill after consumption
- o Consumer died after consumption
- o Other, namely ……

If question 26. was answered with discomforts/ill or death, fill in the symptoms that were suffered (tick all that apply):

Symptoms:

- o Abdominal cramps
- o Diarrhea
- o Bloody diarrhea
- o Vomiting
- o Increased gas
- o Fatigue
- o Muscle ache
- o Loss of appetite
- o Fever
- o Nausea
- o Headache
- o Dizziness
- o Muscle weakness
- o Difficulties in breathing
- o Paralysis
- o Blurred or double vision
- o Slurred speech
- o Death
- o Other, namely……

27. What are the storage conditions you recommend to your customers?

- o Refrigerated/cooled
- o Below room temperature
- o Room temperature
- o Above room temperature
- o Other, namely ……

28. What is the shelf life at the specified storage conditions?

- o <1 day
- o 1-2 days
- o 2-7 days
- o 1 week-1 month
- o >1 month

29. How did you determine your shelf life?

- Tasting
- Visual appearance
- By drinking the product at various stages to check for any adverse effects
- By laboratory testing
- Other, namely……

30. What are the characteristics of a spoiled product?

- Strange color
- Strange smell
- Acidic taste
- Bitter taste
- Alcoholic taste
- Mould on product
- Presence of bubbles or foam
- Change in viscosity
- Other, namely……

In-depth interviews

These guiding questions were used in an in-depth interview with various bigger producers that would have the potential to upgrade their production.

- What do you define as good quality of your product and how would you ensure that your product's quality is obtained and maintained?
- What do you think proper hygiene is?
- Are there any steps that you give extra attention to when producing munkoyo/chibwantu? What do you do now to ensure the safety of your product?
- Do you believe your way of producing munkoyo/chibwantu could be improved concerning the hygiene?
- If you want to upscale your production, would you take subsequent steps to ensure its quality and safety? What measures would you take?
- What is your idea of a quality/safety plan? Do you think it is necessary?
- Have you ever heard of HACCP or ISO? What do you know about them? Would you implement such a plan in your company or prefer to stick to your own way of working?

Interview 1

What: interview with Mildred of C&M Holdsworth
When: 6/1/18
Topic: in-depth questions on knowledge about hygiene

What is quality: maintaining taste standards, having good hygiene and using transparent bottles so people can see the product (the color must be right), our brand: it stands for quality.

How to achieve good quality: We have a manager that monitors the process: all steps must be performed properly. The maize is cleaned, the surroundings are clean. At the moment, we spend so much on all of this, that we are probably not even making a profit, but building up a name is more important at the moment. Therefore we want to perform tests, like the pH for example, but also to check if the products are clean. We also want to obtain a manufacturing license; therefore we will be checked by a health institution. With their permission we can obtain a certificate, which we can show on our label. The ZABS certificate might not even be that useful. The quality is maintained by our commitment, we want to become big, but still we rather produce small amounts than make mistakes. So far no one came by to check on the hygiene, so trust of the consumer comes from delivering a good product. They will spread the word. That way marketing is also not needed.

Improvements
- Commercial, big pot to make bigger amounts. Although it might be difficult to clean. You might need chemicals. Can you still call it natural though? More automated could be cleaner, but is it better?
- Closing of bottles using machine, perhaps also filling, although the pipelines can get dirty.
- Try to reduce the manual handling. We know the people that work for us though, and we trust them.

Do you have any idea about what a hygiene plan is or should be like? Have you ever heard about HACCP or ISO?

I saw one in a restaurant, but people can't always read. I instruct my workers orally. If we were to go industrial, we'd need to document our practices, have a manual on general hygiene. Also we would have checkpoints in the process to check for contaminations, currently we have minimal instruments. I would like to make the plan myself. My cooks should have a medical check each year. I have never heard of HACCP, but I have heard about ISO. Small scale practices are usually not checked in Zambia though. For export they are. We do not have a hygiene plan at the moment, but we

discuss everything with our employees and check them all the time. We teach them to wash their hands, wear gloves and wear a clean suit every 2-3 days. Their own clothes need to be clean every day. We'd like to maintain a pH standard, around 4. This is easy to measure and doable with machinery that we have access to at this point.

Interview 2

What: interview with Otis of Traveller's Kitchen
When: 17/1/18
Topic: in-depth questions on knowledge about hygiene

What is quality?
- Customers get the taste that they expect.
- No one should get ill

How to achieve the best quality:
- Best hygiene practices possible during production, storage and consumption
- I'd like to put warning on the label about how one should treat the product
- We assigned a quality manager and check everything ourselves as well. The manager tastes, inspects visually, checks for foreign objects in the drink and controls the hygiene

The workers have a routine They shower before work, they get everything explained orally and are fired if they do not comply with our rules. I already had to fire people in the past. Especially now that there's cholera, I am extra careful. Even the bottles were a problem once: there was oil in them. We now wash all our bottles prior to filling. We would like to clean them with chlorine as well. We now fill the bottles with a funnel.

We would improve the cooking process if possible. Make it electric, automatic and have a bottle filler. We would like to improve the surroundings to reduce the risk of cholera contamination or spreading. We need to divorce from traditional practices to become more sterile. Also, I'd like to have air-conditioning. Funding is difficult though, all these things cost money.

The bottles we use are transparent to make people trust us more. Some producers try to hide their products by using opaque bottles, but that doesn't make people trust your product. The product should be brown, not black.

I would love to have an ISO-plan. Currently we work orally to teach our workers about food safety. Furthermore I pray every day. Also, our workers cannot come to work when they are ill. I prefer rural employees, they think more than the urban ones.

Interview 3

What: interview with the sister in law of Sydney
When: 22/1/18
Topic: in-depth questions on knowledge about hygiene

What is quality and how do you achieve it?
Use only water, roller meal, good roots, sugar and a calabash. You need a big cooking stick and fire. The timing needs to be right! Adding the roots too early, late or little result in sour munkoyo. You need good hygiene and fine ingredients. Storage should be dry and not allow insects to come into the raw materials. Wash your hands, make sure that the tools are clean. Water should be luke warm when added. The munkoyo should become brown, not black.

If we had more money, we would first want to get our own bottles and labels. Also we would like to have a different fermentation vessel, that consumes less time than the calabash. However, buckets are even slower. A faster fermentation would be great.

I have never heard of a food safety plan, but I think it would be best if one would using paintings or drawing, since many people can't read.

Interview 4

What: interview with Kabwe lady
When: 24/1/18
Topic: in-depth questions on knowledge about hygiene

What is quality and how do you achieve it?
The munkoyo should be brown and thick, but not very thick. It needs a certain, specific taste. I maintain quality by cooking the mealie meal properly, the porridge must never be undercooked. I use the best munkoyo roots. Also I make sure that the surroundings I work in are clean, kept well swept and mopped. Food should be covered at all times and if I had workers, they would have to look clean. I always guard the munkoyo, so no one can do something to it. Currently, with the cholera outbreak going on, I am not producing. If one person gets ill and says it's because of me, it can ruin my whole business.

Next to keeping everything clean, I boil all water that I use. I don't know what I would do with more money, I like it the way it is right now. I do not think a food safety plan is necessary. You just produce a few times, see how it goes and from there on people should trust you. If I would make a plan I would use drawing or cartoons.

Consumer quantitative questionnaire

Question	Answer	Respondents (%)	Respondents (n, if multiple answers possible)
Which product do you consume?	Munkoyo	28.3%	
	Chibwantu	20.8%	
	Both	50.8%	
How often do you consume munkoyo/chibwantu?	Daily	16.7%	
	Weekly	41.7%	
	Monthly	31.7%	
	Yearly	9.2%	
What production method do you prefer?	Traditional	84.2%	
	Commercial	7.5%	
	No preference	8.3%	
Where do you get your <u>traditional</u> munkoyo/chibwantu?	I make it myself		40
	Local market		21
	Mini-mart		6
	Filling station		1
	Supermarket		2
	From friends or family		52
	Neighborhood		12
	Other, namely…….		11
Is munkoyo safe?	Yes	59.2%	
	Yes, as long as you know how to prepare it properly	25%	
	No	13.3%	
	Do not know	2.5%	
What risks do you associate with traditional munkoyo/chibwantu?	No risks		70
	Unhygienic cooking tools		19
	Black magic/added poison		3
	Wrong munkoyo root		16
	Unhygienic fermentation tools		16
	Wrong storage of raw materials		1
	Packaging		14
	Dilution of root extract		6
	Dilution of munkoyo		5
	Wrong storage of finished product		2
	Other		18
How do you make sure for yourself that the <u>traditional</u> munkoyo/chibwantu that you drink is safe?	Obtain it from a familiar source		79
	Make it myself		37
	Check storage conditions		14
	Check production conditions		17
	Check product visually		31
	Smell product		4
	Ask producer questions		3
	Other		24
Which one do you think is safer to consume?	Traditional	30.8%	
	Commercial	40%	
	Both as safe	29.2%	

Do you drink the bought traditional munkoyo/chibwantu immediately?	Yes	15.8%	
	No	29.2%	
	Both	53.3%	
	No response	1.7%	
How do you store your munkoyo/chibwantu?	Room temperature	25%	
	Refrigerator	60%	
	Cool place	0.8%	
	Hole in the ground	0.8%	
	Drink immediately	10%	
	No response	3.3%	
When do you stop drinking munkoyo/chibwantu?	Bitter	18.8%	
	Sour	22.2%	
	Alcoholic	23.1%	
	Slimy	3.4%	
	Never throw away	32.5%	
Do you know what food poisoning is?	Yes	48.3%	
	No	40.8%	
	No response	10.8%	
If you or anyone else you know has experienced discomfort after consuming traditional munkoyo/chibwantu, what do you think was the reason for this?	Wrong root used		23
	Overfermented		18
	Unhygienic cooking tools		11
	Unhygienic fermentation tools		9
	Black magic/added poison		3
	Wrong storage of finished product		7
	Wrong packaging of product		9
	Dilution of root extract		2
	Dilution of munkoyo		1
	Other		17

Table 7 All answers consumer questionnaire

Producer quantitative questionnaire

Question	Answer	Respondents (%)	Respondents (n, if multiple answers possible)
Do you burn the porridge?	Yes	436.7%	
	No	53.3%	
How long do you boil the porridge?	<1 h	20%	
	1-2 h	46.6%	
	2-3 h	20%	
	>3 h	13.3%	
At what temperature do you add the munkoyo root?	Directly after boiling	13.3%	
	When it is still slightly painful to touch	26.7%	
	Luke warm	46.7%	
	Body temperature	6.7%	
	Room temperature	6.7%	
Do you cover the container during cooling?	Yes	60%	
	No	40%	
How long is the fermentation?	8 hours	66.7%	
	24 hours	20%	
	No response	13.3%	
What kind of fermentation container do you use?	Plastic bucket	60%	
	Calabash	26.7%	
	Other	13.3%	
Do you cover the fermentation container?	Yes	100%	
	No	0%	
Do you sieve the end product?	Yes	33.3%	
	No	53.3%	
	No response	13.3%	
What is your target group?	Friends/family	20%	
	Commercial	80%	
What ingredients do you use?	Mealie meal		12
	Maize grits		5
	Whole munkoyo root		6
	Munkoyo root extract		9
	Flour		2
	Sugar		11
When do you add sugar?	I do not add sugar	15.4%	
	After fermentation	53.8%	
	Other	30.8%	
At what temperature do you store your raw materials	Room temperature	100%	
How do you know that the product is ready to drink?	Tasting		8
	By looking		3
	By measuring pH		0
	Other		4
How do you stop fermentation?	By transferring the product to another container		5
	By cooling the product		7
	By boiling the product		0
	I do not stop fermentation		4

Do you standardize anything in the process?	No		2
	Time		2
	Temperature		0
	pH		0
	Cups to weigh amounts		9
	Scale to weigh amounts		1
	Other		1
How do you protect your raw materials?	I do not protect them		3
	Cat		3
	Traps for rodents		0
	Repellant		8
	Other		1
Do you ever throw away ingredients?	No		4
	Mouldy roots		2
	Mouldy mealie meal		1
	Rodent faeces in ingredients		1
	Smelly water		1
	Insects in ingredients		5
	Odd-looking maize		1
	Other		3
What root do you use?	White, dry	30.8%	
	White, fresh	7.7%	
	Yellow, dry	38.5%	
	Yellow, fresh	15.4%	
	Other	7.7%	
Where do you get the root?	Market	69.2%	
	Pick them myself	15.4%	
	Other	15.4%	
What do you pay attention to when picking the roots?	Colour		8
	Shape		4
	Smell		3
	Taste		3
	Other		6
Can the roots be dangerous?	Yes	100%	
	No	0%	
What symptoms can you get from the wrong root?	Abdominal cramps		3
	Diarrhoea		11
	Vomiting		9
	Fatigue		1
	Fever		1
	Nausea		1
	Headache		1
	Dizziness		1
	Death		4
	Other		5
How do you clean the roots?	By hand		7
	Using water		6
What is the source of the water you use	Borehole	30.8%	
	Pipeline	61.5%	
	Other	7.7%	
Do you boil the water prior to use	Yes	53.8%	

	No	30.8%	
	No response	15.4%	
Do you dilute the munkoyo after cooking?	Yes	38.5%	
	No	38.5%	
	No response	23.1%	
How do you clean the tools?	Cold water		10
	First boil water, then use it		1
	Soap		9
	Bleach/disinfectant		3
	Other		4
Why do you throw the finished product away?	I never do		5
	Mould on product		2
	Insects in product		3
	Too sour		6
	Too alcoholic		6
	Other		6
What measures do you take to ensure proper production?	Wash hands		11
	No jewellery		5
	Wear hairnets		10
	Wash tools		12
	Measure time		1
	Check raw materials		10
	Inspect end product visually		4
	Taste end product		1
	Smell end product		2
	Check consistency end product		1
	Clean surroundings		12
	Cool final product		4
	Use disinfectant		1
	Other		5
Did you ever experience anything bad regarding your product?	No complaints		11
	Spoiled product		1
	Other		1
Why did the consumer complain/experience discomfort?	Wrong fermentation		1
Do you know another producer that had a bad experience regarding their product?	Consumer ill		2
	Consumer dead		1
What symptoms did their consumers suffer?	Abdominal cramps		1
	Diarrhoea		1
	Death		1
	Other		1
Why did the consumer complain/experience discomfort?	Wrong root		2
	Wrong fermentation		1
	Unhygienic fermentation tools		1
Recommended storage conditions	Refrigerated	76.9%	
	Room temperature	15.4%	
	Other	7.7%	
Shelf life of product at recommended storage conditions	1-2 days	7.7%	
	2-7 days	38.5%	
	1 week-1 month	15.4%	

	>1 month	38.5%	
How did you determine the shelf life?	Taste	46.2%	
	Drink at various stages	7.7%	
	Other	38.5%	
	No response	7.7%	
What are the characteristics of a spoiled product?	Wrong colour		2
	Wrong smell		3
	Too sour		5
	Too bitter		5
	Too alcoholic		8
	Mouldy		1
	Bubbly/foamy		3
	Wrong viscosity		1
	Other		3

Table 8 All answers consumer questionnaire

Personal experience	Frequency	%
Abdominal cramps	5	27,8
Abdominal cramps, diarrhoea	5	27,8
Diarrhoea	4	22,2
Vomit	1	5,6
Nausea	1	5,6
Dizziness, muscle weakness	1	5,6
Abdominal cramps, diarrhoea, headache	1	5,6
Friend	**Frequency**	**%**
Diarrhoea	14	30,4
Abdominal cramps, diarrhoea	10	21,7
Diarrhoea, vomit	8	17,4
Abdominal cramps, diarrhoea, vomit	3	6,5
Don't know symptoms	3	6,5
Abdominal cramps	2	4,3
Abdominal cramps, diarrhoea, nausea	1	2,2
Death	1	2,2
Diarrhoea, bloody diarrhoea	1	2,2
Diarrhoea, blurred/double vision, nausea	1	2,2
Diarrhoea, vomit, death	1	2,2
Vomit	1	2,2

Table 9 Combinations of symptoms experienced by consumers

Significance tests questionnaires

Test	Compared between/within	What	Significance	Significant?	Degrees of freedom
Fisher's exact test	Education level	Is munkoyo safe?	0.352	No	7
	Age respondent		0.759	No	5
	Residence		0.257	No	2
	Education level	Knows what food poisoning is	0.000	No	7
	Age respondent		0.041	No	5
	Residence		0.257	No	2
	Education level	Which one is safer	0.004	No	7
	Age respondent		0.223	No	5
	Residence		0.073	No	2
	Education level	Preference	0.619	No	7
	Age respondent		0.941	No	5
	Residence		0.676	No	2
ANOVA	Product group	Boiling time	0.597	No	3

Table 10 Statistical tests and their significance chapter 3