RISK ASSESSMENT OF A TRADITIONAL ZAMBIAN DRINK

Master thesis GEN, FHM, FQD

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ABSTRACT

Mabisi, a fermented milk drink, is one of the traditional beverages from Zambia. The product is important as food source for many inhabitants, young to old. Regardless the importance, no safety standards and regulations are yet made to protect health of the consumers. A risk assessment is indispensable to secure consumers health. To make this assessment, three activities were designed and executed: (1) determining the safety perception of consumers and producers, (2) monitoring of the presence of pathogens and resilience of the product against selected pathogens in the product and (3) consideration and implementations to conduct a safe product.

In this study we found that there is a market in the urban regions of the country for a safe traditional product. Purchase behaviour of urban citizens was linked to availability and safety concerns. The majority of the producers (8/9) saw no risk in production of traditional mabisi with concern to the consumers health. And half of all consumers (n=172) interviewed perceived the traditional product as safer compared to the commercial alternative (51%). The majority (60%) associated no risks to the consumption of the traditional product.

Despite that, this study revealed that traditional methods of fermenting raw milk into mabisi in Zambia pose potential hazards to human health. Enterobacteriaceae and S. *aureus* were detected frequently (10 out of 14) in ready to sell products. Most products (9/10) contaminated with S. *aureus* did not reach the infective dose to start an infection. Samples from two producers detected for growth of *Salmonella spp.* with the plate count method and none of the samples detected positive on *B. cereus* or *Shigella spp.*

With challenge tests we found that contamination during fermentation and during storage at refrigeration temperatures resulted in survival of food borne-pathogens. With increased fermentation temperature a decline of the pathogenic growth rate during fermentation was shown.

Most producers have knowledge of critical points in the process, however it is unclear whether these steps receive the actual attention they need. To minimise the risk of food borne diseases, some approaches could be used. Some of those strategies are: implementation of a starter culture, education on food hygiene and storage for consumers, improvement of Good Agricultural Practices (GAP), Good Manufacturing Practices and (GMP) and Good Hygienic Practices (GHP) for producers and more research on critical control points and their limits.

ABBREVIATIONS

BC	*Bacillus cereus*
BGA	Brilliant Green agar
BHI	Brain Heart Infusion
BP	Baird-Parker
EC	*Escherichia coli*
LB-broth	Luria-Bertani broth
LM	*Listeria monocytogenes*
MRS	De Man, Rogosa and Sharpe
MYP	Mannitol Egg Yolk Polymyxin
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose agar
SA	*Salmonella* spp.
SC	*Staphylococcus aureus*
SS	Salmonella Shigella
TSI	Triple Sugar Iron
VRBG	Violet Red Bile Glucose
GAP	Good Agricultural Practices
GMP	Good Manufacturing Practices
GHP	Good Hygienic Practices

TABLE OF CONTENTS

INTRODUCTION

Throughout Zambia a large variety of fermented products can be found. Fermented beverages are part of the Zambian traditional cuisine and are often made at small scale for household use and consumption by family and friends. Lusaka, the capital of Zambia, has inhabitants originating from various different tribes, each with different traditional foods. Small scale producers are selling their products on the local markets. Till this day no standards and safety rules have been formulated for the traditional beverages and the traditional products are not allowed on the formal market. Since a few years, even large commercial producers make products inspired on traditional foods, although according to many these commercial variants are merely a shade of the traditional product.

Due to tribal and geographical influences different production methods can be found throughout the country. Although these different production methods create a different end product they all use variants of the same name; mabisi.

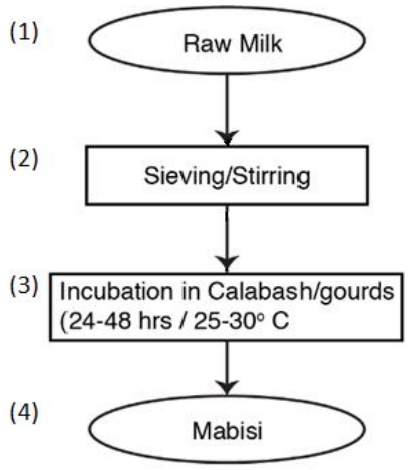


Figure 1 Flow diagram of production process of traditional mabisi

Figure 1 shows a production method of traditional mabisi. Differences in the production method that can be found. Microorganisms inherent in the milk, container and environment ferment the milk in 1-2 days at ambient temperatures. Variations have to do with the draining of the whey, the use of back-slopping, differences in fermentation vessels and the fermentation time.

According to public health organisations each year thousands of people in Africa fall ill because of food borne disease. Major contribution is caused by contamination with

foodborne pathogens (Motarjemia & Kiifersteinb, n.d.; World Health Organization, 1984).

Pathogenic microorganisms of great importance, linked to the consumption of raw milk products, are of zoonotic nature. Zoonoses are defined as diseases and infections transferred from animals to humans. Without causing any apparent illness the cow could be a host of these bacteria. Milking hygiene and farming practices can reduce bacterial contamination, but cannot eliminate it. Examples of pathogens of concern are *Staphylococcus aureus*, *Campylobacter jejuni*, *Salmonella* ssp., *Bacillus cereus*, *Listeria monocytogenes* and *Escherichia coli* (Jayarao et al., 2006; S.P. Oliver, Jayarao, & Almeida, 2005).

In practice, the market places of Zambia lack proper facilities for cold storage and raw milk is not heated once throughout the entire production and logistic chain. During handling and preparations of food, contamination may originate from different sources including food handlers, dirty pots and cooking utensils, flies and pests, polluted water, dirt, domestic animals and more. Especially during food preparation there is a high risk of cross contamination. Together with time (insufficient fermentation time) and temperature (storage conditions) abuse during food preparations this can lead to foodborne diseases.
Education and awareness of producers and consumers about these risks can prevent a major part of the cases related to foodborne diseases (Motarjemi, 2002; Nout & Motarjemi, 1997).

Some say that lactic acid fermentation can improve safety of a food source due to changing environmental composition of the initial product (Hammes & Tichaczek, 1994). For the specific case of mabisi, several questions arise when considering the application of fermentation as the biggest safety guard against foodborne diseases (Dalu & Feresu, 1996; Ogwaro, Gibson, Whitehead, & Hill, 2002). Firstly, what is the consumer perception and awareness on perceived risk, food related hazards and hygienic practices associated with traditional fermented mabisi (Chapter 1)? How resilient is the microbial community of mabisi against invasion of pathogens (Chapter 2)? And thirdly, what considerations and implementations should be taken into account in order to successfully produce a safe mabisi (Chapter 3)?

CHAPTER 1

CONSUMER AND PRODUCER PERSPECTIVE ON SAFETY CONCERNING MABISI PRODUCTION AND CONSUMPTION

INTRODUCTION

The Republic of Zambia is a sub-Saharan African country which is landlocked and shares borders with eight neighbouring countries: the Democratic Republic of Congo, Tanzania, Malawi, Mozambique, Zimbabwe, Botswana, Namibia, and Angola. Zambia has a land area of 752,614 km^2 (approximately twice the size of Germany) of which only an area of 1.23% is covered by water (9,220 km^2).
Rapid urbanization throughout developing countries, as well as for Zambia, has been identified as a major problem related to foodborne diseases and outbreaks. Zambia's population was estimated to be about 16 million people in 2015 with a high growth rate of 3.06% (based on United Nations World Population Prospects). The capital city, Lusaka, situated in the south-central part of the country on an elevated level of 1277 metres above sea level is most densely populated. With high migration rates from rural areas to urban locations, next to new economic opportunities, also problems of poor hygiene and sanitation, accumulation of waste, poor drinking water and deteriorating environmental conditions do occur. Under these conditions food preparation and food handling are exposed to an increased risk of cross contamination. Education and awareness of these risks could prevent many cases of food borne diseases.

Raw milk products and their consumption are linked to bacterial outbreaks and associated with food borne illnesses and diseases. Traditional production of mabisi is mostly done in the rural places of Zambia. The raw milk is spontaneously fermented without any prior heat treatment. Producers of traditional mabisi are mostly women of farmers with access to milk, market sellers which buy their milk or home producers. Since there is limited access to fresh raw milk in the capital most consumers of the traditional drink obtain the product on the market. At the market place traditional mabisi is sold under low hygienic standards. No standardisation or regulations are applied there.
In Lusaka, the capital city of Zambia, a commercially produced product called 'lacto mabisi' is available in the supermarkets. This product is made of pasteurized milk and is standardised. However this product characteristics are not near the traditional mabisi

This paper attempts to answer what the consumer perception and awareness is on perceived risk, food related hazards and hygienic practices associated with traditional fermented mabisi and its storage with use of questionnaires. Besides consumers, hygiene perception and critical points in production for food safety of several producers were analysed.

The overall question in this chapter is;
What is the consumer/producer perception and awareness on perceived risk, food related hazards and hygienic practices associated with traditional fermented mabisi?
The next sub questions will be answered:

- What is the consumers preference, when differentiating between commercial and traditional mabisi, and why? Is there a difference in consumer demand between the commercial and the traditional drink?
- Are consumers aware of risks involved with raw fermented milk?
- Are consumers aware of good storage practices concerning mabisi?
- Are people getting sick due to consumption of mabisi and what are the related symptoms?
- Is there a difference between demographic data and the questions above? (Appendix 1)

It was expected that consumers in the capital, Lusaka, generally prefer traditional over commercial but consume the traditional one less often due to lack of availability. Furthermore, awareness on foodborne illness related to mabisi was expected to be low due to education levels.

METHOD

PROCEDURE

First a trial consumer questionnaire was completed by twenty respondents (Appendix 1). All interviews followed the same structure but were open for elaboration on answers. These were reviewed and the experience was used to design a second trial questionnaire. The improved version had a different wording, different types of questions and additional questions to broaden the perspective. The second trial was again completed by twenty respondents after which it was reviewed and rectified into the final questionnaire, which can be found in Appendix 1.

The producer interview was conducted by information on the different production methods, open interviews with producers and literature on HACCP.

The answers from questionnaires were coded and analysed with IBM SPSS statistics processor and RStudio. Using Frequency tests and the Chart Builder frequency, percentages and significance were obtained. Fisher exact test and Chi-square were used to statistically test for significant associations between groups.

PARTICIPANTS

CONSUMER

A total of 172 questionnaires were held combined over three location, Lusaka (n=108), Choma town (n=32) and rural Choma (n=32). Respondents were recruited by means of spontaneous face to face interaction. All respondents participated on a voluntary basis. Respondents only requirement was that they once had consumed traditional mabisi.

PRODUCER

Nine producers were interviewed (n=9) on their production method and their hygiene perception. This included producers from Choma (n=7) and near Lusaka (n=2).

RESULTS AND DISCUSSION

CONSUMERS

Questionnaires were taken in Zambia (n=172). Of all questionnaires 37 were translated, thus not taken in English. The sample of 172 questionnaires were composed of 111 males (64,5%) and 61 females (35,5%). With 120 subjects (70%) employed and others as unemployed, student or retired. Major group of education level was secondary level (39%), which implies O- or A-level (GCE). From all subjects most belonged to the Tonga tribe (43%). Main age groups were distributed as 20-29 (42%), 30-39 (22%), 40-49 (21%).

SAFETY PERCEPTION

Of all subjects 75% (n=129) generally preferred traditional mabisi over the commercial available product (n=43), and 59% purchased traditional mabisi more often (Table 1). Concerning safety, 88 subjects (51.2%) answered that the traditional drink was safer than the commercial alternative. Most subjects, 135 (78.5%), perceive traditional mabisi as a safe product in general.

Table 1 shows education level against preference, purchase and safety perception. Trend is shown when setting education against preference. Higher level of education shows increase in preference of commercial mabisi. However not significant ($p=0.25$), this could be because unequal distribution of classes. Association between purchase and safety perception against education level are significant (Fisher exact test $p<0.05$). Higher level of education is correlated with lower purchase of the traditional drink and lower safety perception.

Table 1 Cross table of highest level of education against preference/purchase/safety of traditional or commercial mabisi (n=172). Last column shows results from all subjects. Lowest rows contains data on if one thinks traditional mabisi is safe in general against education. Data obtained from surveys and analysed in SPSS. Significance of Fisher exact test is indicated with (*).

		What is your highest level of education?					
		No education	Primary	Secondary	Tertiary	University	Total
Preference	Traditional	100,0%	80,8%	76,1%	78,8%	62,5%	75,0%
	Commercial		19,2%	23,9%	21,2%	37,5%	25,0%
Purchase*	Traditional	83.3%	76.9%	64.2%	57.6%	37.5%	59.3%
	Commercial	16.7%	23.1%	35.8%	42.4%	62.5%	40.7%
Safer*	Traditional	50,0%	80,8%	61,2%	30,3%	32,5%	51,2%
	Commercial	50,0%	19,2%	38,8%	69,7%	67,5%	48,8%
Traditional safe*	Yes	100,0%	92,3%	82,1%	72,7%	65,0%	78,5%
	No		7,7%	17,9%	27,3%	35,0%	21,5%

In addition, education level showed association with knowledge on food poisoning (Fisher exact p=0.00) and illness related to the drink, however no association was found between education level and knowledge on lactose intolerance (p=0.23) (Appendix 1A, Table 12).

Two questions in the questionnaire were scaled by the participant, importance of hygiene and hygienic practices at selling locations of mabisi. On a scale from 1 (not important/ not hygienic) to 10 (important/ hygienic) how important is hygiene for the participant and how hygienic is the production at your location of purchase. Due to high standard deviations no correlations between age, gender, education and residence was found with answers on hygiene. From all subjects a mean value of 8.88 ± 1.96 was given for hygiene importance and a 6.44 ± 2.84 for hygienic practices (n=172). Thus people value hygiene, however they are not convinced producers adopt the same high standards for hygienic practices. No correlation was found between the two scales (Cronbach's alpha < 0.7). Due to high standard deviation no significant difference between hygiene importance and hygiene of selling place can be observed (Appendix 1A, Table 14).

Association between contingency tables of purchase and preference were found (Pearson Chi square <0.05). Subjects who have a preference for traditional mabisi are more likely to think it's safe in general (88%). Next to this 64% thinks it is safer than the commercial product. And 64% does not think it can cause any illness. Of the people that prefer commercial mabisi still 49% perceives traditional mabisi as safe in general, 12% thinks traditional is safer than commercial (n= 5) and 49% does not think it can cause illness (n=20) (Appendix 1A, Tables 15 & 16 & 17).
From the subjects which perceive traditional mabisi as safer compared to the commercial product (n=88), 3 cases answer that traditional mabisi is not safe in general. Only one subject answers that there are bacteria in traditional mabisi that can make you sick and is discouraged by cholera. The other two see no risk of illness in the product, however are discouraged due to hygiene of the seller and selling place.

Motivation of purchase comparing purchase of traditional and commercial products can be seen in Figure 2. Taste and personal preparation of product have highest counts for purchase of traditional mabisi. For commercial mabisi highest counts were observed for availability and safety (Appendix 1A, Table 18).

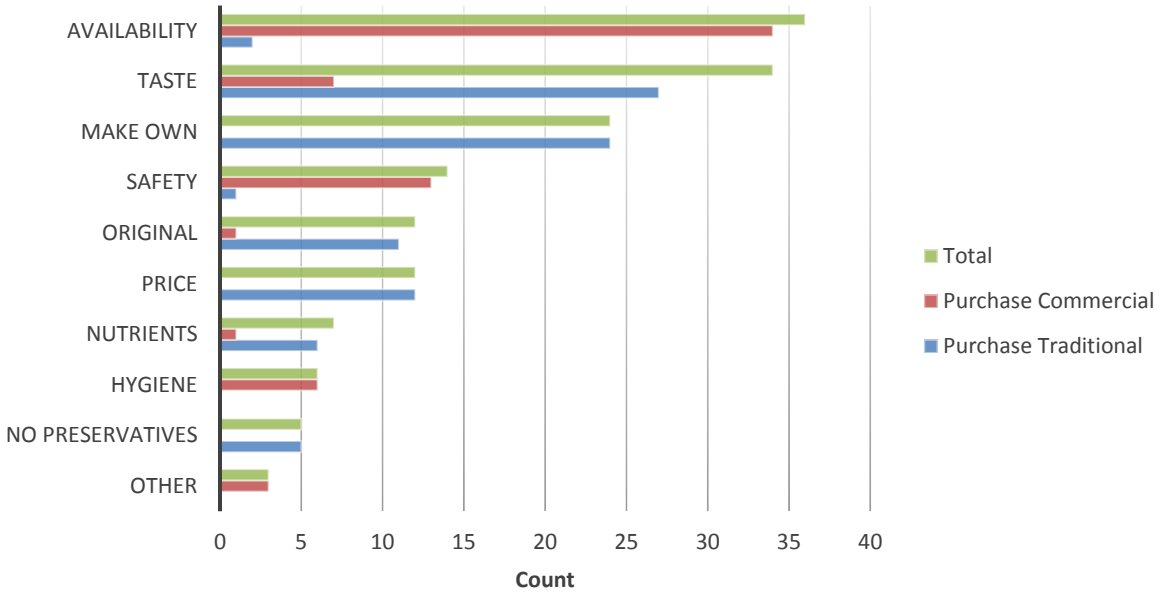


Figure 2 Reasoning behind purchase comparing between purchase of traditional buyers and commercial buyers. Data obtained from SPSS frequencies. Codes made for grouping.

ILLNESS

One of the known syndromes related to milk products is lactose intolerance. Only 12 of all the cases (n=172) had knowledge of lactose intolerance. Of all cases 25% (n=48) had experienced illness in general after consuming fresh milk (untreated cow's milk). In 10 cases, illness after consumption of fresh milk was always experienced, from which eight had symptoms such as stomach ache and diarrhea or both. There is a possibility that these participants are lactose intolerant, but were not aware of this fact. Only 3 out of 10 cases experienced illness from mabisi as well. From the 10 subjects, 2 had any knowledge on lactose intolerance (Appendix 1A, Tables 19 & 20).

Illness after consumption of mabisi was experienced by 25 out of 172 cases. Most cases only experienced it once (n=13) or sometimes (n=9). Illness experienced after consumption of traditional mabisi had a higher count (n=20) compared with illness after the commercial product (n=5).

From the cases that experienced illness linked to mabisi (n=25) still a majority perceives traditional mabisi as safe in general (n=16). When choosing between traditional and commercial, safety perception is that commercial mabisi is safer (n=16). While experienced illness, 10 subjects perceive no risk of illness related to traditional mabisi. From the 15 cases that do perceive risk, 67% relate bacteria to this problem. When asked if one is discouraged by anything during purchase, 18 answer with yes from which most are discouraged by hygiene of seller, place or storage (Appendix 1A, Tables 21 & 22). The perception of food safety and risks by consumers, in general, strongly depends on psychological interpretations. The psychological interpretation of product properties, such as safety perception of the product, is of greater influence than the physical properties on food choice (Yeung & Morris, 2001). Apparently this is also applies to the buying of mabisi.

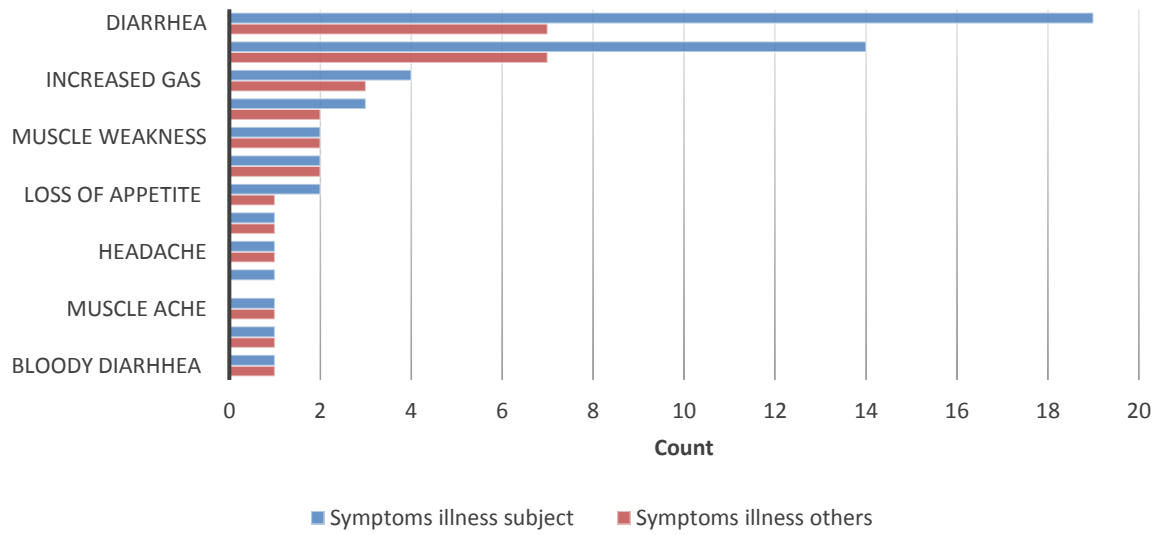


Figure 3 Symptoms related to illness of mabisi by subject themselves or from others they know. Data split in people who experienced illness (n=25) and who know someone who experienced illness (n=30). Data analysed in SPSS.

Figure 3 shows symptoms that subjects related to illness caused by consumption of mabisi by experience or by hearing of. Highest counts are found to be diarrhoea and increased gas, which are symptoms often related to food poisoning. Symptoms from own experience and others are similar.

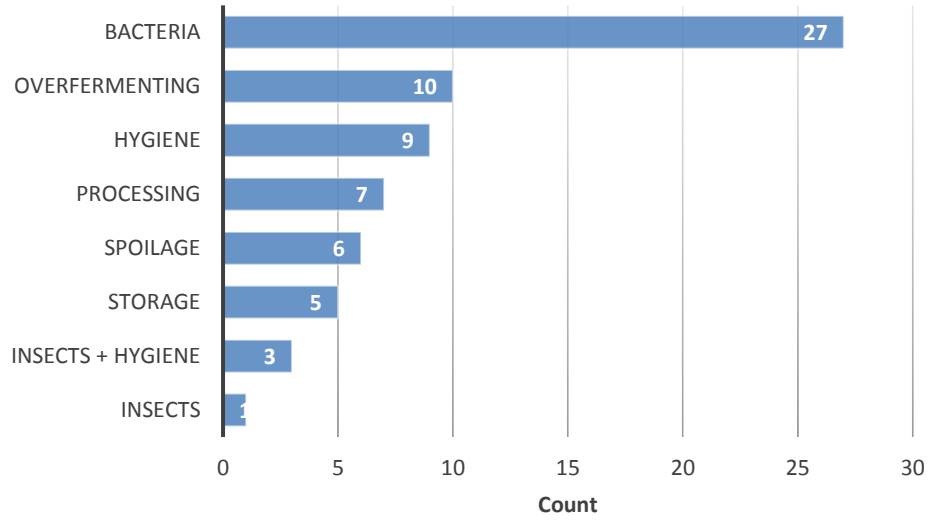


Figure 4 Reasoning behind risk linked to consumption of traditional mabisi. Data split by subjects that relate risk with consumption (n=68). Data analysed with SPSS.

Data samples were selected to analyse the perceived risk of illness related to consumption of traditional mabisi (n=68). Thus most subjects (60%) do not perceive any risk related to consumption of the product. Figure 4 shows reasoning behind this relation. The major group (n=27) relates the risk to bacteria, followed by over-fermenting (n=10).

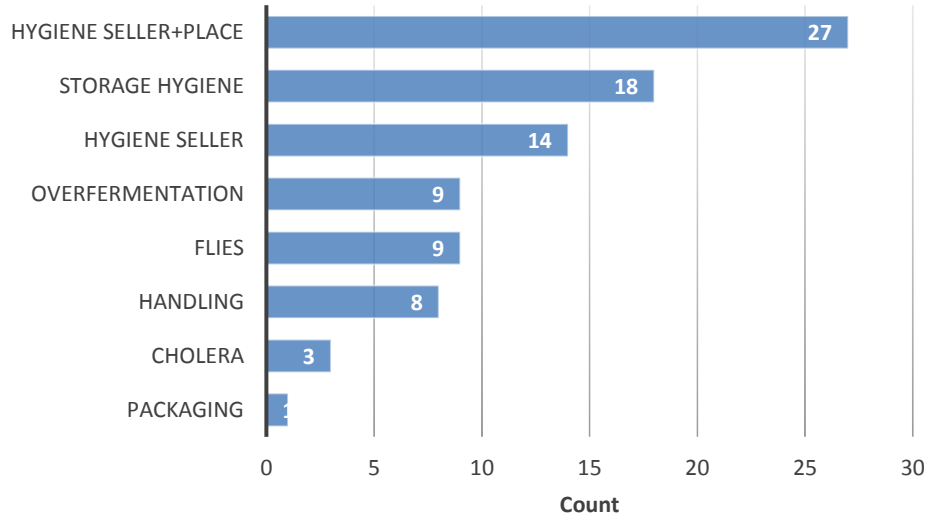


Figure 5 Reasoning behind discouragement. File selected on subjects discouraged when buying traditional mabisi (n=89). Data analysed in SPSS.

When buying the product 89 (52%) of all subjects (n=172) is discouraged when buying mabisi. The reasoning attributes of discouragement are shown in Figure 5. Most people are discouraged by hygiene of seller, place and storage.

STORAGE PRACTICE OF CONSUMERS

After purchase people store their mabisi in the fridge (n=89), at room temperature (n=43) or do not store it at all (n=38). When refrigerated, 57% stores the product for 1-2 days, followed by 26% who store it for 3-4 days. When kept at room temperature 63% stores it for 1-2 days and 35% for 3-4 days (Appendix 1A, Table 23).
Main reasons to stop consumption of the product are bitterness and acidity. Cases that stored their product at room temperature have a higher percentage (30%) of stopping of consumption due to acidity compared to cases that store at refrigeration temperature (12%). This could be due to continuation of the fermentation process and its formation of organic acids (Appendix 1A, Table 24).
There is no correlation found between purchase location, storage conditions (time and temperature) and reason to discard.

SOCIO-ECONOMICAL CLASS

Questionnaires were taken in Lusaka (capital) and Choma (southern village). Within Lusaka distinction could be made between two social classes: poor people from compounds in Lusaka and middle class people of Lusaka.

Table 2 contains results of the questions when this distinction is made. Appendix 1A, Table 25 contains counts on this data.

Table 2 Cross table of residence against preference/purchase/safety of traditional or commercial mabisi (n=172). Lowest rows contains data on if one thinks traditional mabisi is safe in general against residence. Data obtained from surveys and analysed in SPSS. Significance of Fisher exact test is indicated with (*).

		Residence			
		Choma	Lusaka	Lusaka compound	Total
Preference	Traditional	84,6%	68,5%	70,6%	75,0%
	Commercial	15,4%	31,5%	29,4%	25,0%
Purchase *	Traditional	86,2%	45,2%	38,2%	59,3%
	Commercial	13,8%	54,8%	61,8%	40,7%
Safer *	Traditional	66,2%	39,7%	47,1%	51,2%
	Commercial	33,8%	60,3%	52,9%	48,8%
Traditional safe	Yes	86,2%	75,3%	70,6%	78,5%
	No	13,8%	24,7%	29,4%	21,5%

Results show that the purchase of traditional mabisi is higher in Choma (86%), compared to the capital. We found a higher preference count and a higher safety ranking for the traditional product. Possible reason for higher safety perception of traditional mabisi over commercial could be due to access point. Sixty percent of the

cases from Choma make their own mabisi or obtain it from family (n=39), while Lusaka citizens and compound residents obtain their mabisi most of all at market places (38% & 44%)
Consumption is more frequently on a daily base for subjects from Choma (45%) compared to from Lusaka (10%) and compound (15%)(Appendix 1A, Table 26). Association was found between contingency tables of purchase behaviour and residence (p=0.00) and between tables of safety perception (traditional/commercial) and residence (p=0.007). Tables can be found in Appendix 1A, Tables 25 t/m 28.

In Table 3, based on Appendix 1A, Table 29, all subject data are split into residence, preference and purchase. Major reasoning explanations behind this purchase are given in percentages. From this table one observes subjects from Lusaka, which prefer traditional but purchase commercial (n=45) due to availability reasons (79%). And preference of commercial product in the city (Lusaka n=31) is due to availability (36%) and safety (33%) reasons.

Table 3 Split file of all subjects into residence (Choma, Lusaka), preference (traditional/commercial), purchase (traditional/commerical) given in percentages. Data obtained from SPSS analysis.

Choma/Lusaka	Preference	Purchase	Most important reason of purchase (%)	
Lusaka	Traditional	Traditional	Taste	44
		Commercial	Availability	79
	Commercial	Traditional	-	-
		Commercial	Availability	36
			Safety	33
Choma	Traditional	Traditional	Make own	42
		Commercial	-	-
	Commercial	Traditional	Price	50
			Make own	50
		Commercial	Taste	33

In Choma no subjects were found which preferred traditional mabisi and purchased the commercial product. The small case group that preferred commercial over

traditional, bought commercial (n=6) due to taste (33%) or bought traditional (n=2) due to price or personal production.

No association was found between place of residence and knowledge on food poisoning (p=0.697), lactose intolerance (p=0.586), preference (p=0.073), illness linked to mabisi consumption (p=0.695) and view on safety of mabisi in general (p=0.132).

PRODUCERS

Qualitative interviews were taken with producers (n=9) of traditional fermented mabisi on production methods and hygiene perception. Producer M1-M5 are farmers surrounding Choma, M6 is a mabisi producer of a Milk Collection Centre (MCC) in rural Choma, M7 is a mabisi producer of a MCC in Choma centre and M8 and M9 are farmers surrounding Lusaka. Table 4 contains data on processing steps, hygienic steps and critical control points obtained from the interviews with the producers.

Table 4 Producers (M1-M9) and their traditional fermented mabisi process. With abbrev. (RT) room temperature, (P) plastic bucket, (MC) milk can, (W) warm water, (C) cold water, (WS) warm water and soap, (CD) cold water and disinfectant and (CB) cold water and bleach (N/A) not applicable. (±) indicates at times.

	M1	*M2*	*M3*	*M4*	*M5*	*M6*	*M7*	*M8*	*M9*
Capacity	5-15 L	<5 L	16-30 L	5-15 L	16-30 L	>50 L	>50 L	>50 L	5-15 L
Sieving of raw milk	+	+	+	+	+	-	+	+	+
Boiling	-	-	-	-	-	-	-	-	-
Starter culture	-	-	-	-	-	-	-	-	-
Back slopping	±	-	-	-	-	-	-	-	-
Fermentation place	RT	RT	RT	RT	RT	RT	RT	RT	RT
Fermentation vessel	P	P	P	P	MC	MC	P	P/MC	P
Fermentation time	24h	24h	24h	48h	<24h	48-72h	48h	<48h	24h
Fermentation time cold	48-72h	24h	48-72h	72h	72h	120-168h	<120h	<48h	<48h
Draining whey	-	±	-	-	-	-	-	±	-
Sieving end product	-	-	-	-	-	-	-	-	-
Fermentation stopped	Fridge	-	-	-	-	-	Fridge	-	-
End check by	Visual	Taste	Visual	Visual	Visual	Visual	Dip stick	Visual	Visual
Sugar addition	-	-	-	-	-	-	-	-	-
Own cows	+	+	+	+	+	-	-	+	+
Storage before use	-	-	-	-	-	+	+	-	+
Udder cleaning	W	C	C	W	WS	N/A	N/A	C	Other
Mastitis check	+	+	+	+	+	+	+	+	+
Sick cow separation	+	+	+	+	-	N/A	N/A	+	+
Repellent	-	-	-	+	+	-	-	+	-
Discarding raw milk	+	+	-	-	+	+	+	+	+
Vessel cleaning	WS	WS	CS	WS	CD	CD	CD	WS	WS
Utensils cleaning	WS	WS	C	WS	CD	CB	CB	WS	WS
Discarding mabisi	-	+	+	-	+	-	+	+	+
Dilution with water	-	-	-	-	-	-	-	-	-
Dilution with raw milk	-	-	-	-	-	-	+	+	+
Complaints	-	-	-	-	+	-	+	+	-

The two MCC's M6 and M7 get their milk delivered by farmers. This milk should arrive sieved by the farmers themselves, however before production of mabisi, M7 sieves with filter paper to be sure that unwanted particles stay behind. The remaining producers sieve with either a tea strainer or a squared strainer, which only eliminates big particles (insects, dung, etc.).

Reasons for discarding raw milk were after positively testing on mastitis, if it was odd looking or containing dung and insects. Some producers only sieved out insects and dung and still used the milk. Reasons for discarding end product were insects and taste attributes. None of the respondents mentioned insufficient fermentation or spoilage as a reason to discard the final product.

If the same fermentation time is followed but lower fermentation temperatures (cold season) are applied, insufficient fermentation could occur and result in survival of food borne pathogens. Most producers tend to increase fermentation time during cold season, with the exception of producers M2 and M8.
Cleaning of the udder, by producer M9, was not done with water but by letting the calve suck and afterwards cleaning with the cow's own tail.

All producers said to be checking on mastitis, however most didn't do the test themselves but performed the test at the MCC. It needs to be noted that mabisi production is mostly done by farmers when no milk is brought to the MCC, so no mastitis test is taken prior to production.

Sometimes undesired product properties (too sour, too thick) of the end product are altered by producers by use of dilution. Diluting the end product with new raw milk could pose a serious risk to human health. Dilution could cause for contamination of pathogens, when insufficient fermentation time is followed. Producer M9 ferments for 3 more hours after addition of milk before selling and producer M7 ferments for half a day extra. Whereas producer M8 does not incorporates any additional fermentation prior to selling.

In general the producers were very generous in the description of the production process. However, as no empirical assessment was performed in this study, there is some doubt whether the actual performance by the producers in the production process is similar to their statements. Such as cleaning of the udder, testing on mastitis and cleaning of fermentation vessel and utensils.

Questions related to hygiene perception of producers on mabisi production were incorporated into the interview. With the exception of producer M8, none of the producers recognised any risk relating to consumer safety associated with production of mabisi. Producer M8 noted that improper handling and hygiene practices could pose a risk for consumers. Because producers perceive no risk in the production process of traditional mabisi, they, when asked, relate hygiene concerns and improvements to livestock farming aspects. Key themes related to proper hygiene were good personal hygiene, good milking practices, clean equipment and clean environment. Most attention in the fermentation process was paid to sieving, coverage of fermentation vessel, storage and hygienic practices. Points that needed improvement in hygiene stated by the producers were milking practices, processing room and animal conditions. According to the producers, the production of a safe product relies on covering the fermentation vessel, milk quality and hygiene of milking and equipment used. Considering upscaling of the process, producers would mainly change milking practices and animal shelter. Complaints that some producers received were based on product properties, such as thickness and taste.

CONCLUSIONS

CONSUMERS

From all subjects (n=172) preference in terms of taste lays with traditional mabisi (75%) when differentiating between commercial and traditional mabisi. However 22% from these subjects buy commercial mabisi (all residents of Lusaka) due to limited availability.

Half of all subjects perceives traditional as safer compared to commercial (51%), and 80% perceived traditional mabisi as a safe product in general. The majority (60%) related no risks to the consumption of the traditional product. The perceived risk by the group that perceived risk (40%) mainly related risks to bacteria (n=27).

The subjects that are discouraged during purchasing (52%) link this to hygiene of seller, place and storage.

Overall, 25% (n=48) had experienced illness after consuming fresh milk (untreated cow's milk). From which ten cases always experienced illness connected with lactose intolerance related symptoms. Illness experienced after consumption of mabisi was lower than of fresh milk, 15% (n=25), with highest counts of the symptoms diarrhoea and increased gas.

Storage practices of the main group consisted of refrigeration (n=89) for 1-2 days (n=51).

Furthermore, the level of education was associated with purchase behaviour and safety perception. Associations were found between contingency tables of purchase and residence and between residence and whether the subject experienced the traditional or commercial product as more safe.

PRODUCERS

The process of making traditional mabisi is based on feeling, tradition and knowledge received from others, not on standardisation and testing. Most (8/9) producers see no consumer risks associated with their production of traditional mabisi. Most have knowledge on critical points in the process, however it is unsure whether these steps receive the actual attention they need.

CHAPTER **2**

PREVALANCE AND INVASION OF PATHOGENIC BACTERIA IN TRADITIONALLY PROCESSED MABISI

INTRODUCTION

In general fermentation is thought of for creating a safe product. Complex microbial communities create an unsuitable environment for pathogens to invade. Lactic acid fermentation is said to be sufficient to kill pathogens due to acidic environment created by lactic acid bacteria (Hammes & Tichaczek, 1994). However, new studies found that fermentation is not necessarily safe and multiple factors have influence on survival of pathogens (Abee, Krockel, & Hill, 1995; Charlier, Even, Gautier, & Le Loir, 2008; Dahiya & Speck, 1968; Nout & Motarjemi, 1997).

Different studies detected pathogens in traditionally fermented milk drinks (Akabanda & Glover, 2010; Dalu & Feresu, 1996; Feresu & Nyati, 1990; Lore, Mbugua, & Wangoh, 2005; Nyatoti, Mtero, & Rukure, 1997). Pathogenic microorganisms of great importance are of zoonotic nature. Without causing any apparent illness, the cow could be a host of these bacteria. Milking hygiene and farming practices can reduce bacterial contamination, but cannot eliminate it. The traditional way of mabisi production and handling is not done under optimal hygienic circumstances and chance of contamination is high. Therefor it is important to detect the prevalence of food borne pathogens in mabisi which could come from the environment, handlers and animals. Pathogens of concern associated to milk based products are *Staphylococcus aureus*, *Salmonella* ssp., *Bacillus cereus*, *Listeria monocytogenes* and *Escherichia coli* (S.P. Oliver et al., 2005; Rohrbach, Draughon, Davidson, & Oliver, 1992).

Variables of interest that could affect the invasion ability of pathogenic bacteria into a fermenting community are fermentation temperature, timing of contamination and cell concentration of the contaminating microbe. Temperature of fermentation during traditional production can differ from day to day. Therefor it is necessary to know if fermentation temperature has influence on survival of relevant pathogens. Contamination can occur before fermentation but as well after fermentation by addition of new raw milk, environment or handling manners.
Also believed is that farmers dilute their milk with water to sell more and therefor earn more money, this study wants to show the impact of dilution on pathogenic survival.

In this chapter research is done on prevalence of pathogenic bacteria in traditional mabisi from different producers with use of qPCR and the plate count method. Expected is that most samples will initially be free of pathogens due to inactivation of pathogens by the low pH of the drink. However contamination from the environment, food handler and handling later on in the process could change these outcomes.

Next to this resilience of the microbial community against pathogenic invasion is tested under several conditions. Difference is made between contamination of the raw material before fermentation (survival during fermentation) and contamination of the finished product (survival during storage). Other questions answered in this chapter are;
Is there difference in survival between pathogens during fermentation and storage?
Is there difference in survival of pathogens between a fermentation temperature of 25ºC and 28ºC?
Is there an impact on survival of pathogens when diluting the milk with 20% (v/v) water?

Expected is that there is a difference between pathogens, because of their difference in optimum growth conditions (temperature, water activity, pH, atmosphere), outer membrane (Gram-negative, Gram-positive) and difference in reaction with other hurdles. Survival of pathogens during storage is expected to be lower compared to survival during fermentation. Degradation rates (d-values) of microorganisms are expected to be higher for environment with low pH (during storage) as well as for the higher fermentation temperature (28ºC). Assumed is that dilution with water will not affect the survival of pathogens in significant ways due to the buffering capacity of bovine milk.

MATERIAL AND METHODS

SAMPLING METHOD

Mabisi samples were obtained from producers and traders on markets (n=14) in Zambia. Nine producer samples from Lusaka (n=2) and Choma (n=7), and five trader samples from the market in Choma (n=4) and Kitwe (n=1). Raw milk samples were collected from all producers from Choma (n=7). Quick pH check was done on the spot. Samples were collected in a sterile tube (duplicate) immediately stored on ice and kept on ice until fridge was available or laboratory (range of time interval of storage on ice between 30 min and 8 hours).

BACTERIAL STRAINS

The bacterial strains used in this study are given in Table 5.These strains were collected from Food Microbiology culture collection at Wageningen University. Stock cultures were frozen at -80 ^{0}C and reactivated on brain heart infused agar (BHI) at 37^{0}C.

Table 5 Bacterial strains obtained from Wageningen University and their selective plating agar used in experimental designs.

Pathogen	Strain ID	Selective Agar
Listeria monocytogenes	Li0001	Brilliance Listeria agar
Salmonella enterica	Sa0222	Brilliance Salmonella agar
Bacillus cereus	Ba0076	Mannitol Egg Yolk Polymyxin Agar (MYP)
Escherichia coli K12	Ec0016	Macconkey agar
Staphylococcus aureus	Sc0108	Mannitol Salt Agar (MSA)

Division of three different experiments can be made.

1. Detection of foodborne pathogens in mabisi with PCR
2. Detection of foodborne pathogens in mabisi with plate count method
3. Resilience of the microbial community against pathogenic invasion

Both detection methods were performed on samples obtained from mabisi producers and traders. Mabisi samples for experiment 3, the resilience of the microbial community on pathogenic invasion, were prepared in the laboratories of Wageningen University.

DETECTION OF FOODBORNE PATHOGENS IN MABISI WITH QPCR

EXTRACTION OF BACTERIAL DNA FROM MABISI SAMPLES

All samples (triplicate) were spun down at 12000 x g for 5 minutes. Supernatant was removed and pellets were stored frozen.

Cells were re-suspended in a mix of 64 µl 0.5M EDTA, 160 µl Nuclei Lysis solution (Promega), 5 µl RNase (10 mg/mL), 120 µl fresh lysozyme (10 mg/ml) and 40 µl fresh pronase E (20 mg/ml). Followed by incubation for 60 minutes at 37 degrees rpm 350. 400 µl ice-cold ammonium acetate 5M was added and gently mixed. Samples were cooled on ice for 15 minutes. Spun down on high speed (13000 x g for 2 minutes). Supernatant (700 µl), containing the DNA, was transferred to a clean 1.5ml micro-centrifuge tube. Equal volume (700 µl) of phenol (=tris-saturated Phenol-Chloroform-Isoamyethanol 24:25:1), was added and vortexed. Samples were spun down at 13000 rpm 4°C for 2 minutes. Supernatant (450 µl) was transferred to a clean micro-centrifuge tube (avoid aspiration of the interlayer or organic phase). Equal volume of Chloroform (450 µl) was added to supernatant, vortexed and spun for 2 minutes 12000 rpm at 4°C. Supernatant (400 µl) was transferred to a clean micro-centrifuge tube mixed with 2-isopropanol (500 µl) vortexed and precipitated overnight (-20°C). After precipitation samples were spun down for 15 minutes at 13000 rpm 4°C. Supernatant was carefully poured out and pellet was washed with 1 mL cold 70% Ethanol. Followed by centrifuging 10 minutes at 12000 rpm 4°C, pouring out supernatant and air drying for 10 min at room temperature. The DNA is dissolved in 20 µL of TE buffer (pH 8.0) and incubated at 37 ^{o}C for 30 minutes.

DNA EXTRACTION POSITIVE CONTROLS

DNA extraction for positive controls *S. aureus*, *E. coli*, *Shigella*, *B. cereus*, *S. enteric* and *L. monocytogenes* was done. No positive control for *Escherichia coli* O157:H7 and Vibrio cholera was done. Pathogens were inoculated in LB-broth and incubated overnight at 37 C. 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) without use of lysostaphin for *S. aureus*. *S. aureus* was there for treated with microbeads for 30 seconds. For all Gram-positive bacteria 20 µl mutanolysin (4 U/µl) was added.

QUALITATIVE AND QUANTITATIVE ASSESSMENT OF DNA

All samples were analysed by spectrophotometry using a Nanodrop spectrophotometer (Bio-Tek instruments, inc.). DNA concentration(A260) and purity(A260/280) were determined by absorbance. The quality of a selection of extracted DNA was evaluated by gel electrophoresis. The sample (2 µl) mixed with a dye and known concentrations of lambda DNA were loaded into the gel to evaluate quality of the isolated DNA.

PRIMERS

The primers were based on sequence data published by Wang, Cao, and Cerniglia, (1997) (Wang, Cao, & Cerniglia, 1997).

Pathogen	**Target gene**	**PCR primers' sequences(5'-3')**	**Product Size**
Listeria monocytogenes	hemolysin gene	LM-1, CGGAGGTTCCGCAAAAGATG	234 bp
		LM-2, CCTCCAGAGTGATCGATGTT	
Salmonella spp.	invA gene	Sal-3, TATCGCCACGTTCGGGCAA	275 bp
		Sal-4, TCGCACCGTCAAAGGAACC	
Bacillus cereus	hemolysin gene	BC-1, CTGTAGCGAATCGTACGTATC	500 bp
		BC-2, TACTGCTCCAGCCACATTAC	
Escherichia coli	malB promotor	Eco-1, GACCTCGGTTTAGTTCACAGA	585 bp
		Eco-2, CACACGCTGACGCTGACCA	
Staphylococcus aureus	nuclease gene	SA-1, GCGATTGATGGTGATACGGTT	276 bp
		SA-2, CAAGCCTTGACGAACTAAAGC	
Shigella sonnei	ipah gene	Shi-1, CTTGACCGCCTTTCCGATAC	610 bp
		Shi-2, CAGCCACCCTCTGAGAGTA	
Escherichia coli O157:H7	hlyA gene	O157–3, GTAGGGAAGCGAACAGAG	361 bp
		O157–4, AAGCTCCGTGTGCCTGAA	
Vibrio Cholera	Toxin gene	VC-1,GGCAGATTCTAGACCTCCT	563 bp
		VC-2, TCGATGATCTTGGAGCATTC	

QPCR

PCR mixture containing 50 mmol L^{-1} Tris-HCL (pH 8.5), 20 mmol L^{-1} KCL, 3 mmol L^{-1} $MgCl_2$, 0,05% bovine serum albumin, 0,25 mmol L^{-1} of each dATP, dTTP, dCTP,dGTP (dNTP mixture), 0,25 µmol L^{-1} of each primer and 0,9 U of Taq polymerase was made. A 2 µL portion of DNA extract, samples and positive controls, was added to 23 µL PCR mixture.
Thermal cycling was carried out using Biorad T100 Thermal Cycler. Denaturation, annealing and extension temperatures were 94, 50, 74 ºC. Denaturation temperature was maintained for 15 seconds during the first cycle and for 3 seconds (94 ºC) followed by annealing for 10 seconds (50 ºC) and extension of 35 seconds (74ºC), for the subsequent 35 cycles. Ending with one cycle of 74ºC for 2 minutes and 45ºC for 2 seconds.

The PCR products were mixed with a dye and separated by electrophoresis in a 1% agarose gel containing ethidium bromide.

DETECTION OF FOODBORNE PATHOGENS IN MABISI WITH PLATE COUNT METHOD

Pre-enrichment of all samples before detection and enumeration was done for viable organisms, enterobacteriaceae, lactic acid bacteria (aerobic/anaerobic), yeast and moulds, indole production, *Bacillus cereus* and *Staphylococcus aureus*. Mixture of 25 grams of product and 225 ml of Buffered Peptone Water (BPW, Oxoid CM0509) was made. The mixture was left at room temperature for 1 hour. Ringer's solution was made with Ringers tablets (Merck, 115525) and subsequently used for serial sample dilutions.

Aerobic plate counts of viable organisms were done by the pour plating method on plate count agar (PCA, Oxoid), followed by incubation at 21 °C for 48 h; colonies were recorded as CFU/mL. Enterobacteriaceae were enumerated on violet red bile glucose (VRBG, Oxoid CM1082) agar by the pour plate method with an over-lay, followed by incubation at 35 °C for 24-48 h.
Lactic streptococci were enumerated on M17 (Oxoid, CM0785) agar enriched by 10% w/v lactose solution (Oxoid, LP0070) by pour plating of the serial dilutions. Plates were incubated at 35 °C for 24-48h.
Mesophilic anaerobic lactic acid bacteria were enumerated with de Man, Rogosa, Sharpe (MRS, Oxoid CM0361) agar. Pour plate method with over-layer was used to create anaerobic environment. Plates were incubated at 37°C to enumerate mesophilic lactic acid bacteria.
Enumeration of yeast and mould were done with spread plating serial dilutions on Potato Dextrose agar (PDA, Oxoid CM0139) and incubation at 21 °C for 5 days. Colonies were checked for characteristics with use of microscopy.

Bacillus cereus was enumerated on Mannitol Egg Yolk Polymyxin (MYP, Oxoid CM0929) agar supplemented with polymyxin B supplement (Oxoid SR0099) and 5% v/v Egg yolk emulsion (Oxoid, SR0047). Agar was poured into sterile petri dishes and the 20 µL per adequate dilution of sample was plated by the drop technique (Herigstad, Hamilton, & Heersink, 2001), followed by aerobic incubation for 24 h at 30 °C.

Indole production was detected using Lauryl tryptose broth (Oxoid, CM0451) inoculated by sample followed by incubation of 24 h at 37 °C in a water bath. After incubation 3-4 drops of Kovacs Reagent (Sigma-Aldrich, CAS Number 100-10-7) were added to the tubes (triplicate). Typical indole production reactions were detected.

The detection and enumeration of *S. aureus* was done by plating the serial dilutions with the drop technique onto Baird Parker Agar (BP, Oxoid CM0275) containing egg yolk tellurite emulsion (Oxoid, SR0054) . Inverted Petri dishes were incubated at 35 °C and counts were made after 24-48 h. All suspicious colonies were characterised with the microscope.

Salmonella spp. and *Shigella spp.* were detected in six successive steps. Pre-enrichment in BPW at 35 °C for 24 h, was followed by enrichment of 100 µL in Rappaport–Vassiliadis (RV, Oxoid CM0669) broth incubated at 42 °C for 48 h. The isolation was done on two selective media, brilliant green agar (BGA, Oxoid CM0263) and xylose lysine deoxycholate (XLD, Oxoid CM0469) agar at 35 °C for 24-48 h. Confirmation of suspicious colonies of *Salmonella spp.* and *Shigella* was done on Triple Sugar Iron (TSI, Oxoid CM0277) slants and Salmonella *Shigella* agar (SS, Oxoid CM0099) agar at at 35 °C for 24h. *Salmonella spp.* and *Shigella spp.* were detected on typical reaction and colony characteristics.

Regarding to the results of plate count methods, one should take into account that the quality of materials and media used was not optimal.

RESILIENCE OF THE MICROBIAL COMMUNITY AGAINST PATHOGENIC INVASION

MABISI PREPARATION

Mabisi was made with three different compositions of milk. UHT milk (Milboa, UHT milk), 20% diluted UHT milk (diluted with sterile water) and raw milk (Hooilanden, Wageningen). Starter culture was obtained by propagation of freezer stock; Mumbwa, 1 ml vial was added to 99 mL UHT milk and incubated for 3 days at room temperature.

The three different milk samples of 99 mL were collected in 100 mL Schott flasks and 1 mL of starter culture was added. The caps were unscrewed slightly during fermentation to allow oxygen to enter and produced gasses to escape.

PATHOGEN PREPARATION

Pathogens from Table 5 were obtained from a freezer stock culture and streaked onto Brain Heart Infusion Agar. After 24 hours of growth on BHI-agar, one colony of each pathogen was taken and inoculated in LB broth (Sigma, L3022) . All broths were incubated overnight at 37 ºC. Assumed was that the broth contained log 9 cfu/ml after the overnight incubation. To obtain an inoculation rate of log 6 cfu/ml, 1 ml of the overnight broth was taken and centrifuged at 10000 rpm for 5 minutes. After centrifugation the supernatant was discarded and the pellet was re-suspended in 1 ml UHT milk. For spiking 100 µl of this suspension was used. Plate count agar (PCA) was used to obtain inoculation rates.

PATHOGENIC INVASION DURING FERMENTATION

Over time invasion of pathogens *E. coli* and *S. aureus* in raw milk mabisi was tracked. Samples were spiked at beginning of fermentation (t=0) and subsequently incubated at 25 or 28 °C for 48 hours. At time points 0, 8, 24 and 48 samples were taken and measured on their pH and the abundance of either *E. coli* and *S. aureus* by streak plating 100 µl of the appropriate dilution on their selective media (Table 5). After incubation of the plates the characteristic colonies were counted. The experiment was done in biological triplicate.

Detection of pathogenic growth was done for UHT milk samples and 20% diluted UHT milk samples at a fermentation temperature of 28°C.

A static invasion was done for: *Listeria monocytogenes* (LM), *Bacillus cereus* (BC), *Salmonella spp.* (SA*).* Were samples were spiked at the beginning of fermentation(t=0) and measured after fermentation (t=48) at 25°C. Enumeration was done for raw milk samples, where UHT milk and 20% diluted UHT milk were only detected on growth.

PATHOGENIC INVASION DURING STORAGE

Investigation of pathogenic survival after fermentation was done with raw milk mabisi samples. Samples were spiked after 48 hours of fermentation at 25 °C with one of the following pathogens: *Escherichia coli* (EC), *Staphylococcus aureus* (SC), *Listeria monocytogenes* (LM), *Bacillus cereus* (BC*)* and *Salmonella spp.* (SA). Samples were stored at 4 °C for 2 (96h) and 4 days (144h). Data was obtained by pH measurements and spiral plating 50 µl of the -1 and -4 dilution on selective agar plates. Whole experiment was done in biological triplicate.
Detection of pathogenic growth was done for UHT milk and 20% UHT milk samples after 1 day (72h) of storage at 7 °C.

RESULTS AND DISCUSSION

DETECTION OF FOODBORNE PATHOGENS IN MABISI WITH PLATE COUNT METHOD

Table 6 shows presence of the pathogens *B. cereus*, *S. aureus*, *Salmonella spp.*, *Shigella spp.* and indole producers in traditional fermented mabisi obtained from producers and traders in Zambia. Examples of indole producers are *Escherichia coli*, *Klebsiella oxytoca* and *Citrobacter koseri*.

Table 6 Results specific plating (log cfu/mL) of *B. cereus* (MYP), *S. aureus* (BP), *Salmonella spp.*, *Shigella spp.* and indole production for producers M1-9 (n=9) and traders T1-5 (n=5). White cells are <2.0 log CFU/mL, grey cells express cell growth (SA) or positive reaction Indole producer (Kovacs reaction) or positive colony with salmonella characteristics. Positive test (PT) and negative test results are indicated with NT for the kovacs and lauryl tryptose test.

		B. cereus	***S. aureus***	**Indole producer**	***Salmonella spp.***	***Shigella spp.***
	pH	MYP	BP	Lauryl tryptose	BGA, XLD, SS, TSI	BGA, XLD, SS, TSI
M1	4.36 ± 0.01	<2.0	<2.0	*E. coli*	<2.0	<2.0
M2	4.52 ± 0.01	<2.0	<2.0	PT	<2.0	<2.0
M3	4.56 ± 0.02	<2.0	2.6 ± 0.23	NT	<2.0	<2.0
M4	3.70 ± 0.02	<2.0	<2.0	NT	<2.0	<2.0
M5	4.40 ± 0.32	<2.0	5.3 ± 0.00	PT	<2.0	<2.0
M6	4.26 ± 0.02	<2.0	3.0 ± 0.48	PT	<2.0	<2.0
M7	4.51 ± 0.02	<2.0	3.9 ± 0.21	PT	S. enteritidis /typhimurium	<2.0
M8	4.20 ± 0.05	<2.0	4.2 ± 0.38	*E. coli*	<2.0	<2.0
M9	3.90 ± 0.04	<2.0	<2.0	PT	S. typhimurium	<2.0
T1	4.40 ± 0.13	<2.0	3.6 ± 0.72	PT	<2.0	<2.0
T2	4.38 ± 0.02	<2.0	3.6 ± 0.69	NT	<2.0	<2.0
T3	4.01 ± 0.01	<2.0	2.4 ± 0.29	PT	<2.0	<2.0
T4	4.26 ± 0.02	<2.0	2.8 ± 0.41	PT	<2.0	<2.0
T5	4.45 ± 0.01	<2.0	3.4 ± 0.12	*E. coli*	<2.0	<2.0

None of the samples were detected positive for *Bacillus cereus* (table 6) and *Shigella* spp. However all traders tested positive on *S. aureus* (5/5) which is not that unexpected due to handling manner of the sellers at the market side. The percentage of *S. aureus* found in all samples is 71% (10 out of 14) Appendix 2, table 35. “Less than 1.0 µg of toxin in contaminated food can produce symptoms of illness. This toxin level is reached when S. aureus populations exceed 10^5 per gram” (HSE, 2013).

Although 10 out of 14 test positive for *S. aureus*, the average cell count of all samples is 3.31 ± 0.94 log CFU/mL which is lower than the population numbers to reach toxic level of *S. aureus*. Only trader M5 reached a 10^5 CFU/mL of *S. aureus* in their traditional fermented sample.

Out of fourteen producers ten detected positive for indole producers. Positive reaction found in -1 dilution or -2 dilution, no higher dilutions were detected positive. For M1, M8 and T5, *E. coli* was detected according to ISO 6579, 6785 and 10272 Standards with TSI agar.

Salmonella spp. was found in 2 out of 14 samples, which both were from producers. The results of BGA, XLD, SS and TSI pointed to *S. enteritidis* and *S. typhimurium* in producer M7 and *S. typhimurium* in producer M9. This could pose risk to human health, for some *S. enteritidis* serotypes low cell intake (4-45 cells) can already cause for disease (Government of New Zealand, 2001), for *S. typhimurium* because it could result in typhoid fever. It is not common, but *S. typhimurium* can be found in raw milk (Jayarao et al., 2006).

Producer M4 tested negative for detection of pathogens *B. cereus*, *S. aureus*, *Salmonella spp.*, *Shigella spp.* and indole production. M4 has a pH of 3.70 ± 0.02 which is low compared to other samples. High acidification could be an explanation for these results, contradicting however are the results of M9 with a pH of 3.90 ± 0.04. Obtained data of qualitative interview with producers showed that producer M9 ferments for 24 hours and producer M4 for 48 hours. Inactivation of pathogens increase with time spent in sour environments and level of pH reduction (Whiting, 1993).

Table 7 shows cell count of all viable organisms, Enterobacteriaceae, lactic acid bacteria (aerobic/anaerobic) and yeast and moulds.

High viable counts (PCA) were observed for all producers and traders. Counts were higher than expected so dilution was not done sufficiently for all samples, thus estimation of cell count is given in Table 7.

Enterobacteriaceae provide evidence of poor hygiene, inadequate processing or post-processing contamination of foods. With VRBG plating one can give an indication of food quality and spoilage potential. Producer M4 did not detect positive for the selected pathogens and has a expectedly low cell count for enterobacteriaceae. Remarkably producers M5, M7, M8 test negative for

enterobacteriaceae (Table 7) but test positive for indole production (Table 6). This could be due to working environment and qualities of media and materials used.

Table 7 Cell count (log cfu/mL) of viable organisms (PCA), Enterobacteriaceae (VRBG), aerobic lactic streptococci (M17), anaerobic mesophilic lactic acid bacteria (MRS) and yeast (PDA). Enumerated for producers M 1-9 (n=9) and traders T1-5 (n=5) of traditional fermented mabisi in Zambia.

		Viable organisms	**Enterobacteriaceae**	**Lactic streptococci**	**anaerobe LAB**	**Yeast**
	pH	PCA (log cfu/mL)	VRBG	M17	MRS	PDA
M1	4.36 ± 0.01	>8.5	5.4	5.6 ± 0.23	>8.5	5.4 ± 0.18
M2	4.52 ± 0.01	>8.5	>5.5	6.0 ± 0.13	>8.5	5.4 ± 0.11
M3	4.56 ± 0.02	>8.5	>5.5	4.7 ± 0.35	>8.5	3.8 ± 0.20
M4	3.70 ± 0.02	7.5 ± 0.00	2.8 ± 0.50	4.0	7.3 ± 0.33	5.1 ± 0.00
M5	4.40 ± 0.06	>8.5	<2	>7.5	>8.5	4.1
M6	4.26 ± 0.02	7.6 ± 0.19	5.4	5.9 ± 0.33	7.0 ± 0.01	5.7 ± 0.28
M7	4.51 ± 0.02	7.2 ± 0.19	<2	6.4 ± 0.30	8.5	6.4
M8	4.20 ± 0.05	6.8 ± 0.34	<2	5.7 ± 0.30	>7.5	4.2 ± 0.14
M9	3.60 ± 0.04	>7.5	>4.5	4.9 ± 0.30	>7.5	5.4
T1	4.40 ± 0.06	>8.5	>5.5	6.3 ± 0.04	8.4	4.9 ± 0.13
T2	4.38 ± 0.02	7.6	>5.5	6.4 ± 0.12	8.1	5.5 ± 0.07
T3	4.01 ± 0.01	7.3	>5.5	6.1 ± 0.03	7.4 ± 0.09	6.1
T4	4.26 ± 0.02	>8.5	4.1 ± 0.08	6.1 ± 0.09	>8.5	6.2
T5	4.45 ± 0.01	8.4 ± 0.28	4.5	8.4 ± 0.06	8.5 ± 0.11	4.4 ± 0.21

High numbers of yeast were observed, this is similar to other traditional fermented milk products (Akabanda & Glover, 2010; Lore et al., 2005; Wouters, Ayad, Hugenholtz, & Smit, 2002). High number of yeast can result in spoilage but also contribute to the flavour.

High counts for anaerobic and aerobic lactic acid bacteria found as expected due to low pH values found. For producer M4 lowest cell count for lactic streptococci found and lowest pH observed. This could be due to several reasons, completion of nutrients, inhibiting compounds for LAB, hydrogen ion concentration too high (low pH) and thus limiting growth of LAB (Hutkins, R. W., Nannan, 1993). Another reason could be that the anaerobic LAB strains have an inhibiting effect on the aerobic strains. Lactococci can produce bacteriocins that increase safety by bacterial activity

against close working strains (Wouters et al., 2002).

DETECTION OF FOODBORNE PATHOGENS IN MABISI WITH QPCR

DNA duplicates were prepared on two different days with the same protocol. The agarose gel however shows amplifications for only one of the duplicates runs.

Appendix 2, Figures and Tables 30 t/m 33 contain Agarose gel Electrophoresis pattern of isolated DNA and Nano drop results. Only few amplifications were visible, see Table 8. In total 22 samples were analysed (Producers n=9, Raw milk producers n=7, Traders n=6) only 5 samples showed amplifications, Table 8. All positively tested results from the plate count method should give amplification in the PCR, if correct. However results of the PCR gel and the plating technique did not correlate.

Table 8 PCR detection of 5 food-borne pathogenic bacterial species in 5 samples.

	***Shigella* spp.**	***E. coli***	***Salmonella spp.***
M3	+	+	±
M5	-	+	-
M9	-	+	-
T2	+	+	-
T3	-	+	-

Bacillus cereus, *Listeria monocytogenes* and *Staphylococcus aureus* are not mentioned in Table 8 because no amplification occurred on the positive controls. Lysozyme was used for extraction of the DNA for both the bacteria extraction as the mabisi extraction. Gram positive bacteria are generally more susceptible to lysozyme than Gram negative bacteria due to cell wall composition. Gram negative bacteria in this study, *Salmonella spp.*, *E. coli*, *Shigella spp.* and *Vibrio cholera* should be more difficult to open up (Masschalck & Michiels, 2003). Even Gram positive showed resistance to lysis with lysozyme, by modification of the basic structure. One of those modifications is possession of 0-acetylated peptidoglycan which causes hindrance for lysozyme activity (Clarke & Dupont, 1992; Moynihan & Clarke, 2010). A different protocol should be used to extract DNA for detection with qPCR.

RESILIENCE OF THE MICROBIAL COMMUNITY AGAINST PATHOGENIC INVASION

INFLUENCE OF STARTING MATERIAL ON PATHOGEN INVASION

Three types of milk, UHT, 20% UHT and raw milk, were used to prepare mabisi. Samples were spiked with *E. coli* and *S. aureus*. In Figure 6 cell count of *S. aureus* and *E. coli* during fermentation at 28 ºC can be seen for all three milk samples. Benchmark of log 2 is added as an detection line, from this line no colonies are detected in -1 dilution.

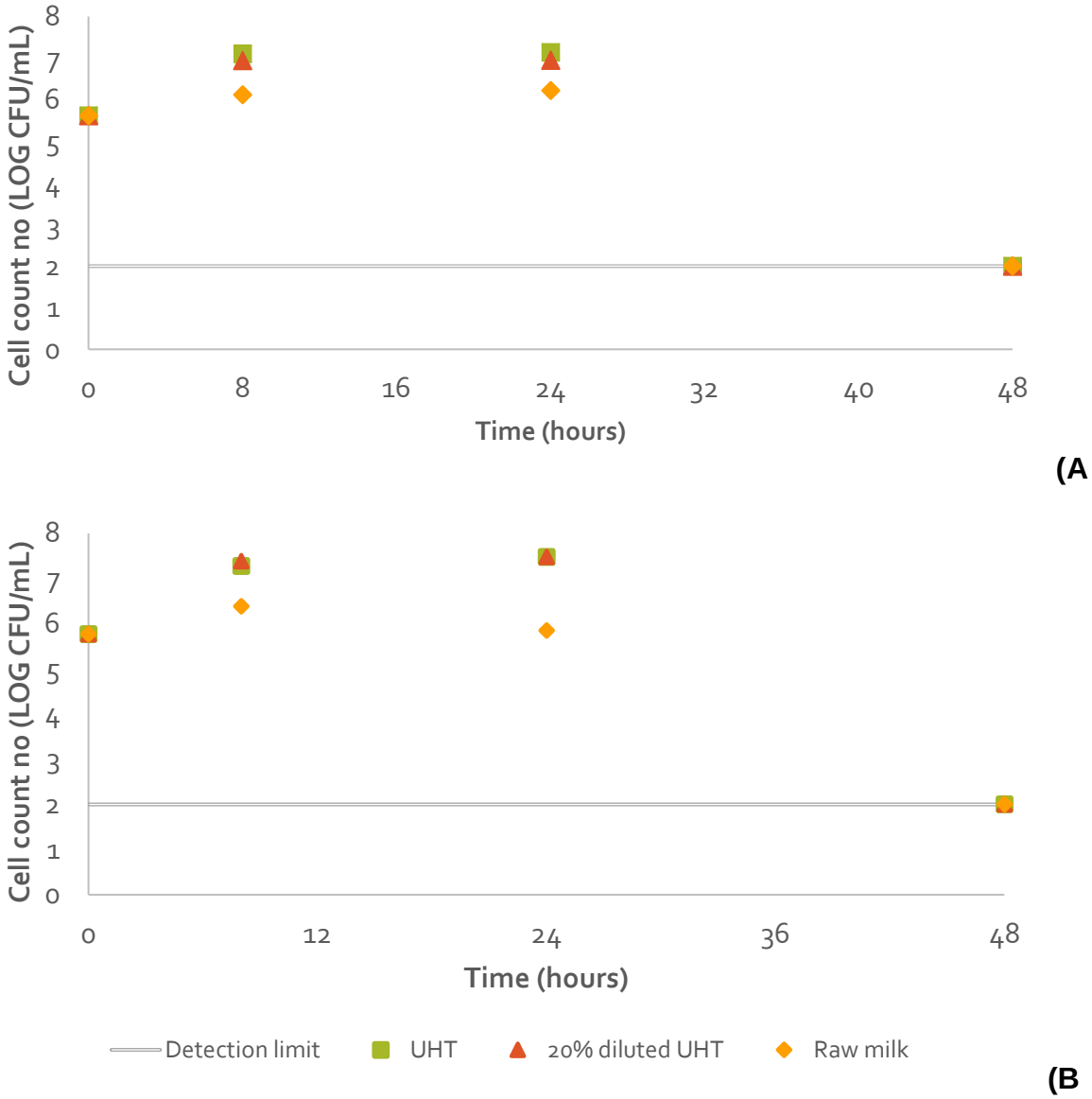


Figure 6 Cell count (LOG CFU/mL) of *S. aureus* (A) and *E. coli* (B) during fermentation of 48 hours at 28ºC for different milk samples, UHT (□), 20% UHT (Δ) milk and raw milk (◊). No detection of colonies resulted in 2 log (given as detection limit). *E. coli* colonies detected on Macconkey agar and *S. aureus* on Mannitol Salt Agar.

Figure 6 (A+B) shows that after fermentation of 28 ºC for 48 hours *S. aureus* and *E. coli* are no longer detected in any of the samples. Comparing Figure 6 (A+B), only one significant difference can be found between *E. coli* and *S. aureus*. This is for UHT milk at time point 24 (p=0.0267) .

Figure 6 (B) shows a significant difference at time point 24 ,between UHT milk and raw milk (p=0.0203), and between 20% UHT and raw milk (p=0.0203). The raw milk mabisi shows an earlier inactivation of *E. coli* and *S. aureus*, between t=8 and t=24. This occurrence could be due to inherent antimicrobial activity of compounds or substrate competition with other bacteria present in raw milk. For example antibacterial activity of lactoferrin due to its iron sequestering property and interaction with the lipopolysaccharide of the Gram-negative membrane (Farnaud & Evans, 2003). As *S. aureus* is known to be a poor competitor with other bacteria this could explain the lower cell count compared to UHT and 20% UHT milk (HSE, 2013). Possibly, the heat treatments of UHT milk could results in inactivation in compounds such as indigenous milk enzymes lactoferrin and lysozyme, (Conesa et al., 2010; Ozturkoglu-Budak, 2018; SÁNCHEZ et al., 1992).

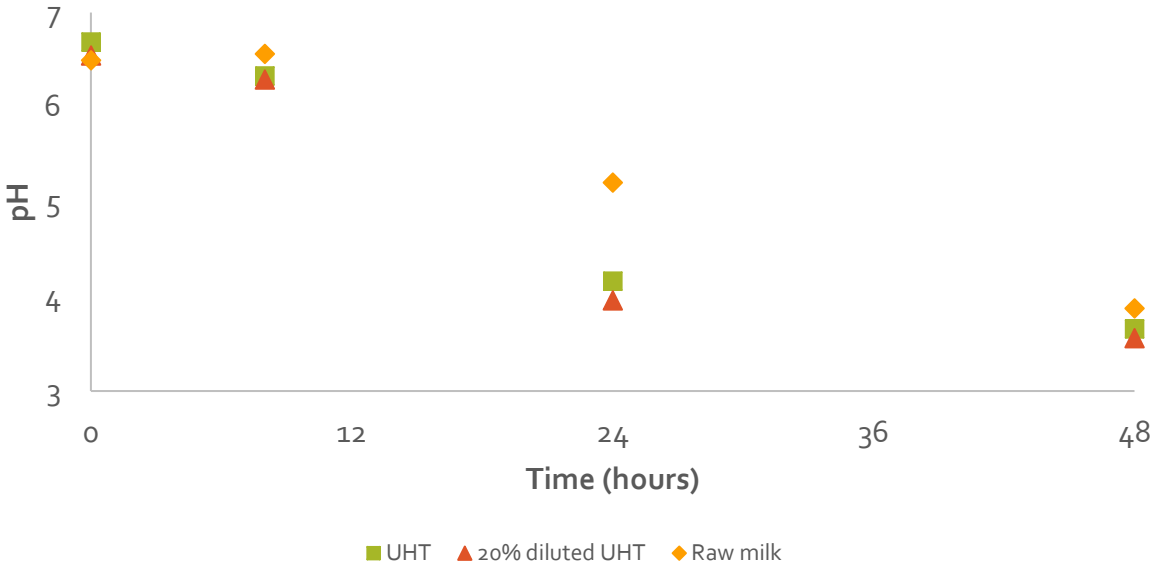


Figure 7 pH of *S. aureus* samples during fermentation of 48 hours at 28^{0}C for different milk samples, UHT (□), 20% UHT (Δ) milk and raw milk (◊). Graph represents results for pH over time for *E. coli* spiked samples and control samples.

No significant differences were found between samples that were spiked with *E. coli*, *S. aureus* or control (not spiked). Therefor Figure 7 is a representative graph for all

three situations. Figure 7 shows a decline in pH with increase of fermentation time. This is expected due to formation of mainly lactic acid produced by lactic acid bacteria and other organic acids coming from the starter culture. Noticeable is the time point of 24 hours where a higher pH of raw milk is found compared to UHT milk ($p=0.0062$) and 20% diluted UHT milk ($p=0.001$). This difference could possibly be linked to the sterile environment for the lactic acid bacteria in UHT-, and diluted milk samples. Less interference of the environment, inactivation of immunoglobulins and lactoperoxidase system who work against LAB, could cause for a faster acidification, thus faster pH drop (Griffiths, 1986).

Expected was that rate of acidification was linked to rate of inhibition (Mufandaedza, Viljoen, Feresu, & Gadaga, 2006), but comparing Figure 6 and 7 at time point 24 raw milk has highest pH (5.21 ± 0.03) and lowest cell counts for *E. coli* (5.85 ± 0.05) and *S. aureus* (6.22 ± 0.27), more than only acid production is related to survival of pathogens. The acids formed, rate and degree of acidification, bacteriocins and other substances formed are to be considered (Feresu & Nyati, 1990; Gadaga TH, 2004; Hammes & Tichaczek, 1994). Some studies found that early antagonistic effects from lactic acid bacteria are not necessarily related to acidification (Charlier et al., 2008; Dahiya & Speck, 1968). Other compounds and substances formed by lactic acid bacteria, such as bacteriocins can prevent spoilage as long as no resistant pathogen is involved (Abee et al., 1995). Furthermore indigenous milk enzymes should be taken into account, such as lactoperoxidase, lysozyme, lactoferrin and immunoglobulins (agglutinins), when raw milk is used (Wheeler, Hodgkinson, Prosser, & Davis, 2007).

INFLUENCE OF FERMENTATION TEMPERATURE ON PATHOGEN INVASION

Raw milk samples inoculated with a starter (inoculation rate of log 5 cfu/ml) were spiked with *E. coli* or *S. aureus* and fermented at 25 ^{0}C and 28 ^{0}C for 48 hours. Even though starting pH raw milk 28 ^{0}C (pH= 6.50 ± 0.007) and 25 ^{0}C (pH=6.67 ± 0.017) are different, pH trend during fermentation only shows significantly lower pH (p=0.0275) for control samples at 28 ^{0}C (pH= 3.85 ± 0.02) compared to 25 ^{0}C (pH= 4.216 ± 0.00) at 48 hours. No other significantly different time points were found between 25 and 28 degrees of fermentation temperature when spiked with *E. coli* and *S. aureus*.

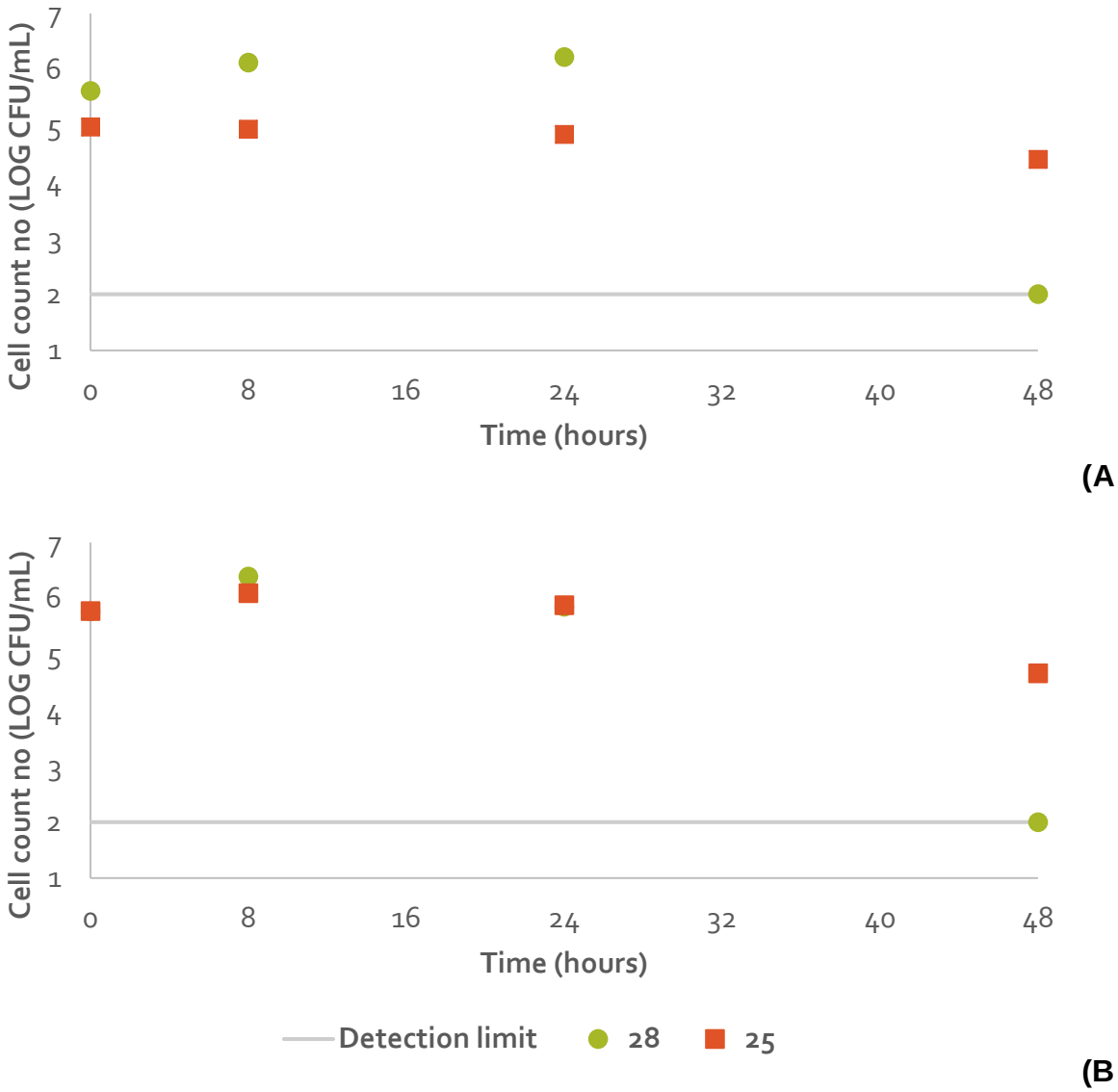


Figure 8 Cell count (LOG CFU/mL) of *S. aureus* (A) and *E. coli* (B) during fermentation of 48 hours at 25 ºC (□) and 28ºC (o) for raw milk samples. No detection of colonies resulted in 2 log (given as detection limit). *E. coli* colonies detected on Macconkey agar and *S. aureus* on Mannitol Salt Agar.

In Figure 8 cell count of *E. coli* and *S. aureus*, comparing two fermentation temperatures, are shown. One can see that at a fermentation temperature of 28 ^{0}C none of the pathogens were detectable after time point 48, while in a fermentation performed at 25 ^{0}C *S. aureus* (4.40 ± 0.230 log cfu/mL) and *E. coli* (4.657± 0.069 log cfu/mL) are still detected.

While 28^{0}C is closer to the optimum growth temperature of *E. coli* and *S. aureus*, which is 37 ^{0}C, no cells are found after 48 hours (HSE, 2013; Park, Worobo, & Durst, 1999). Although the pH was not significantly lower after 48 hours, faster acidification and growth of LAB bacteria and their antimicrobial compounds could be a reason for reaching the detection threshold at 28 ^{0}C and not at 25 ^{0}C. Inactivation of *E. coli* depends on the pH, temperature and acidulant (Park et al., 1999), and *S. aureus* is inhibited by substances produced by lactic acid bacteria (HSE, 2013).

Significantly higher colony count for *S. aureus* was detected for 28 ^{0}C after 24 hours ($p=0.003$) and lower colony count for 48 hours ($p=0.005$). This occurrence after 24 hours could be due to different spiking rates, 25 ^{0}C (4.98 ± 0.064 log cfu/mL) and 28 ^{0}C (5.62 ± 0.005 log cfu/mL) for *S. aureus*.

For samples spiked with *E. coli* only significant differences are seen at time point 48, were 28 ^{0}C has reached the detection limit.

Previous studies also showed survival of pathogenic and non-pathogenic *E. coli* strains during fermentation of unpasteurized milk products at 20ºC and 25ºC for 24h and 48h (Feresu & Nyati, 1990; Mufandaedza et al., 2006). This indicates that contamination of *E. coli* and *S. aureus* in raw milk followed by a fermentation of 48 hours pose an actual risk for consumers, when fermented at 25ºC.

INFLUENCE OF INVASION OF SEVERAL PATHOGENS DURING FERMENTATION

Raw milk, inoculated with a starter (4.70±0.43 log cfu/mL), was spiked with *B. cereus*, *Salmonella spp.*, *L. monocytogenes*, *E. coli* and *S. aureus* and fermented for 48 hours at 25 ^{0}C.

Table 9 Cell count (log cfu/mL) of LM, SA, BC, EC, SC on time point t=0 and t=48. Samples were fermented at 25 ^{0}C. Cell count obtained by spiral plating on specific plate per pathogen.

	0 (h)	**48 (h)**
L. monocytogenes	6.12 ± 0.077	4.74 ± 0.117
Salmonella spp.	5.82 ± 0.040	7.69 ± 0.468
B. cereus	4.99 ± 0.119	3.74 ± 0.039
E. coli	5.77 ± 0.055	4.66 ± 0.069
S. aureus	4.98 ± 0.064	4.40 ± 0.231

E. coli and *S. aureus* results for time points 0 and 48 are added to table 9, to create an overview of all pathogens and their survival during fermentation. None of the pathogens experienced elimination below the detection threshold within the 48 hours of fermentation. Pathogens showed significant decrease in cell count were *L. monocytogenes* (p= 0.002), *B. cereus* (p= 0.049) and *E. coli* (p= 0.002), except *Salmonella spp.* which significantly increased (p= 0.028) and *S. aureus* which showed no difference (p= 0.058). The pH values of all samples can be found in Appendix 2, Table 36. All samples acidified to approximately pH 4.1, which is similar to pH of mabisi found throughout Zambia.

Growth of *Salmonella spp.* during fermentation could be due to acid adaption. Several studies found acid adaptation of *Salmonella spp.*, indicating the presence of a survival or stress resistant mechanism during fermentation of dairy products, slower acidification could contribute to this mechanism (E.A., 1992; Lin, Lee, Frey, Slonczewski, & Foster, 1995). Another explanation could be generally low minimum pH for growth of *Salmonella spp.*, minimum pH of growth is 3.8. Minimum pH is dependent on temperature, presence of salt and nitrite and presence of acids and their type (Robinson, 2014).

Fermentation of 48 hours at 25^{0}C is not efficient to inactivate pathogens, nevertheless causes for decline in *E. coli*, *B. cereus* and *L. monocytogenes*.

INFLUENCE OF INVASION OF SEVERAL PATHOGENS DURING STORAGE

The impact of invasion of *E. coli*, *S. aureus*, *B. cereus*, *L. monocytogenes* and *Salmonella spp.* after fermentation of 48 hours during storage of 2 (96 h) and 4 days (144 h) at 4 ^{0}C is investigated. Raw milk samples were fermented at 25^{0}C for 48 hours and spiked by pathogens.

Table 10 Invasion of pathogen (LM/SA/BC/EC/SC) after fermentation of 48 hours at 25 degrees. Log cfu/ml is obtained by spiral plating on selective plates after 2 and 4 days of storage at 4 degrees. Dark grey indicates no detection of colonies at -1 dilution.

	End fermentation	**Storage**	
	48 h (Log CFU/mL)	**96 h (2 day)**	**144 h (4 days)**
L. monocytogenes	5.07	4.76 ± 0.195	4.77 ± 0.043
Salmonella spp.	5.23	5.19 ± 0.512	4.47 ± 0.297
B. cereus	5.05	<2.0 ± 0.000	<2.0 ± 0.000
E. coli	5.38	5.01 ± 0.046	5.02 ± 0.081
S. aureus	4.97	3.40 ± 0.077	3.37 ± 0.170

After storage at 4 ^{0}C for two days observed is a decrease in *E. coli*, *S. aureus*, and *L. monocytogenes* but not a significant difference between two days and four days in storage. Due to failed replicates for the inoculation rates at time point 48, one cannot tell if this is an actual trend observed.

B. cereus is no longer detected after two days of storage. This inactivation could be due to low pH environment created by lactic acids (Røssland, Borge, Langsrud, & Sørhaug, 2003).

The plate count *Salmonella* ssp. doesn't show a difference within two days of storage (5.19 ± 0.512), but a decrease is found after 4 days of storage (4.47 ± 0.297). However due to high standard deviations the difference between the 2 days and 4 days of storage is not showing significant decrease (p=0.059). All pH values can be found in Appendix 2, Table 37. Significant decrease from 2 to 4 days of storage was observed for *L. monocytogenes*, *Salmonella spp.* And *B. cereus* samples. Slow acidification was occurring due to low storage temperature, same was found in other fermented dairy products when spiked with a pathogen (Tsegaye & Ashenafi, 2005).

Other studies found that storage at ambient temperature resulted in higher survival rates of pathogens than refrigeration temperatures (Dalu & Feresu, 1996; Feresu & Nyati, 1990; Tsegaye & Ashenafi, 2005).

CONCLUSION

Results previously described in this work indicate that traditional methods of fermenting raw milk into mabisi in Zambia pose potential hazards to human health. Enterobacteriaceae and *S. aureus* were detected frequently (10 out of 14) in ready to sell products. However, numbers of cells found for *S*. *aureus* did not often reach toxic level of 1.0 µg which could result in symptoms. This toxin level is reached when S. aureus populations exceed 10^5 per gram

Contamination during fermentation and during storage at refrigeration temperatures resulted in survival of food borne-pathogens. Higher fermentation temperature was shown to decrease pathogenic invasion.

Differentiating between raw material used for fermentation, one found that there was earlier inactivation of *E. coli* and *S. aureus* for raw milk samples. However, dilution of milk with water (20%) did not result in significant differences. Shown was that survival of pathogens was not only influenced by acidic environment, but other factors were contributing to the fate of the pathogen as well.

CHAPTER 3

IMPLEMENTATION AND CONSIDERATIONS OF HAZARD ANALYSIS AND CRITICAL CONTROL POINTS

INTRODUCTION

Documentation of several outbreaks related illness can be traced back to consumption of raw milk (Stephen P. Oliver, Boor, Murphy, & Murinda, 2009). Regulations and hygienic standards are formed, in the United States of America and the European Union, to limit related milk borne illnesses., Zambian Bureau of Standards (ZABS), has regulations for raw milk, yoghurt and other globalized milk products. However the traditional Zambian fermented milk drink, mabisi, is not yet regulated.

Risk assessment is defined as the determination of quantitative or qualitative risk estimation related to a defined situation and a recognized threat. With a risk assessment food safety can be promoted. HACCP (Hazard Analysis and Critical Control Points) is a risk management tool. This chapter will highlight the implementation and consideration of a HACCP system to ensure food safety of traditional operations of mabisi.

In the case of mabisi, which is made from raw milk, food safety is mostly determined by the quality of the raw material, presence of zoonotic pathogens (from cows), spoilage and good manufacturing practices (milking practices and handling).

Good agricultural practices (GAPs), good manufacturing practices (GMPs) and good hygienic practices (GHPs) enhance food safety and quality. If these practices are correctly executed, initial- and re-contamination can be avoided, invasion of pathogenic microorganism can be reduced and/or growth of pathogens can be limited.

Because the traditional milk drink is mainly made on a small scale production or at home HACCP, practices (GAP, GMP and GHP) need to be tailored to the setting of these small-scale producers in rural Zambia. It is important to enhance acceptance and sustainability, due to community preference, socioeconomic factors and to reach maximum results (Amoa-Awua et al., 2007; Jans et al., 2017; Motarjemi, 2002; Nout & Motarjemi, 1997).

SUGGESTED HACCP AND DISCUSSION

Since the production process of mabisi is relatively simple, most food safety is connected to the quality of the raw material and to proper practices. Since Zambia does not have defined regulations for drinks based on raw milk fermentation, European Union regulations are used as a guide. First of all raw milk should come from animals which are healthy and do not show symptoms of infectious diseases. Udders should be normal and udders should not been treated with substances that are likely to be dangerous to human health.

Raw milk must come from holdings with good conditions of animal housing, hygiene, cleanliness and health of animals. Moreover, satisfactory hygiene conditions for milking handling, cooling and storing milk should be applied. Implementation of proper personal hygiene of the milking personnel. Equipment and instruments which come in contact with milk must be made of smooth material which is easy to clean.

Raw milk for fermentation purposes by Regulation (EEC) No 2377/90 must meet the following standards (The council of the European communities, 1992);

Plate count 30º C (per mL)	≤100 000
Somatic cell count (per mL)	≤400 000
*Staphylococcus aureus** (per mL)	n= 5 m= 500 M= 2000 c= 2
Salmonella spp. /Listeria monocytogens**	Absent in 1 gram n= 5 c= 0
Coliforms***	n= 5 m= 0 M= 5 c= 2

n = number of sample units
m = threshold value for number of bacteria.
M = maximum value for number of bacteria.
c = number of sample units where the bacteria count may be between m and M the sample being considered acceptable if the bacteria count is m or less.

*Raw milk product that does not contain heat treatment in their process.

** Other products than cheese and milk powder

*** Liquid milk-based products

When a starter culture is incorporated other law requirements are needed, such as assessment of the suitability and the safety status of the microorganisms of issue and its antimicrobial resistance (Laulund, Wind, Derkx, & Zuliani, 2017).

Suggested HACCP can be found in Table 11 with the corresponding production process Figure 9. This is a suggestion of critical control points that could be found in a HACCP system made for traditional production of mabisi. A detailed system can only be made if the actual production place is defined.

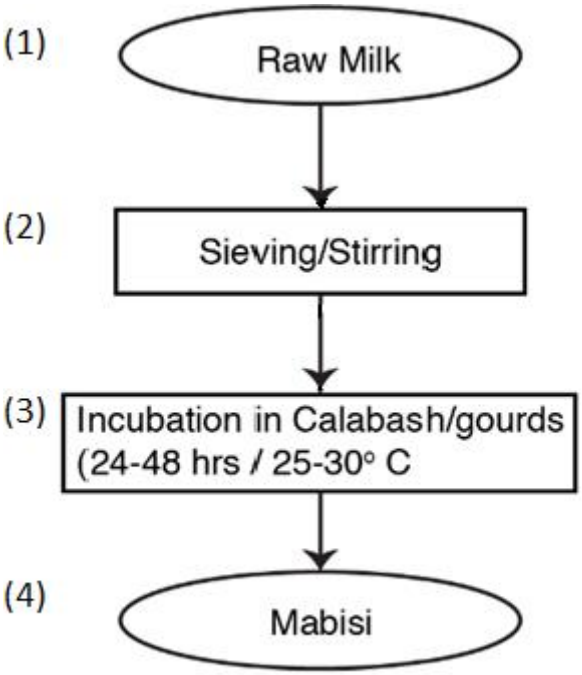


Figure 9 Flow diagram of traditional fermented mabisi production.

The HACCP system is designed for a small production place. It is not designed for home processing. This is due to the materials needed to perform the tests. Home producers should mainly focus on the GAP, GHP and GMP. Actual details on fermentation time, temperature and storage conditions need more research before these can be defined in the HACCP system.

Table 11 Flow diagram for manufacturing of Mabisi, hazards at each steps, CCPs and monitoring.(The numbers in brackets indicate each stage in the manufacturing process).

Step	***Process Step***	**Hazards**	**Control Measures**	**Critical limits**	**Monitoring procedure**	**Corrective action**
1	Raw Milk	Presence of micro-organisms	Microbiological analysis. MBR test	CCP	Plating Test kit	Reject milk if exceeds limits
		Antibiotics	Rosa test Kundrat test ATK test	CCP	Plating/ test strips	Reject milk if exceeds limits
		Mycotoxins and aflatoxins	Rosa test	CCP	Test strips	Reject milk if exceeds limits
		Pesticides	Test	CCP	Test kit	Reject milk if exceeds limits
		Mastitis / abnormal milk	California test Alcohol test	CCP	Test kit	Reject milk if tested positive
		Sanitary condition	Resazurin test	CCP	Analytical	Reject milk if <4
		Abnormal milk	Visual	CCP	Visual	Reject milk if abnormal
2	Sieving	Foreign material	Sieving	CCP	Visual	Additional sieving or rejection
3	Incubation	Presence of dirt in bucket	Cleaning	CP	Visual	Wash
		Insufficient fermentation	pH control Temperature	CP	pH meter/strips Temperature meter	Longer fermentation or rejection
		Foreign materials / pests	Covering lid	CCP	Visual	Reject milk
4	Storage	Improper storage conditions	Temperature	-	Temperature meter	More studies needed

FURTHER RESEARCH AND RECOMMENDATIONS

In Chapter 2 the presence of pathogenic bacteria in mabisi was demonstrated, even though most quantities were harmless the risk should of pathogenic growth should be taken seriously. However, most producers and consumers perceived no risk with the production and the consumption of the product (Chapter 1). Therefore, education of food handlers and food vendors on food hygiene should be one of the prevailing strategies to prevent foodborne diseases linked to mabisi. Consumers should be educated on product handling and storage.

Risk reduction could be gained by producers by improvement of GAP, GMP and GHP. Proper milking practices and cattle handling, proper hygiene, clean environment and clean equipment are only a few examples of importance to diminish the contamination of pathogens. Education and proper practices could take place in the form of a cartoon to overcome the problem of illiteracy.

Next to briefing activities in the domain of food technology a starter culture could be introduced. With the use of a starter culture milk could be heated prior to use. This will reduce the presence of pathogens. However to create a sustainable implementation a starter culture with predominant local fermentative microorganisms should be made. Herewith a product with similar characteristics such as specific flavours and organoleptic properties is maintained. To implement such a starter culture into the process of the daily production of mabisi more attention should be paid to process preferences, education and consumer/producer awareness.

In Chapter 2 presence of pathogenic bacteria was detected in most of the samples which were selected, however from the 172 interviewees only 25 experienced illness. Interesting for further research is if demographics and environmental factors have a large impact on diversity of the microbiome residing in the human gastro-intestinal tract and therefor resilience against food borne pathogens. This could alter critical limits set for substances before causing harm.

To create a defined risk assessment and HACCP for small scale producers more research is required. Such as additional studies on occurrence of pathogens and their fate in mabisi should be performed with a comprehensive view on pathogens of interest. The influence of the fermentation temperature and the fermentation time should be studied to create critical control points. Research on shelf life and storage practices are needed to create a defined risk assessment.

REFERENCES

Abee, T., Krockel, L., & Hill, C. (1995). Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. *International Journal of Food Microbiology*, *28*(2), 169–185. http://doi.org/10.1016/0168-1605(95)00055-0

Akabanda, F., & Glover, R. L. K. (2010). Microbiological Characteristics of Ghanaian Traditional Fermented Milk Product , Nunu. *Nature and Science*, *8*(9), 178–187.

Amoa-Awua, W. K., Ngunjiri, P., Anlobe, J., Kpodo, K., Halm, M., Hayford, A. E., & Jakobsen, M. (2007). The effect of applying GMP and HACCP to traditional food processing at a semi-commercial kenkey production plant in Ghana. *Food Control*, *18*(11), 1449–1457. http://doi.org/10.1016/j.foodcont.2006.10.009

Charlier, C., Even, S., Gautier, M., & Le Loir, Y. (2008). Acidification is not involved in the early inhibition of Staphylococcus aureus growth by Lactococcus lactis in milk. *International Dairy Journal*, *18*(2), 197–203. http://doi.org/10.1016/j.idairyj.2007.03.015

Clarke, a J., & Dupont, C. (1992). O-acetylated peptidoglycan: its occurrence, pathobiological significance, and biosynthesis. *Canadian Journal of Microbiology*, *38*(2), 85–91. http://doi.org/10.1139/m92-014

Conesa, C., Rota, C., Castillo, E., Pérez, M. D., Calvo, M., & Sánchez, L. (2010). Effect of heat treatment on the antibacterial activity of bovine lactoferrin against three foodborne pathogens. *International Journal of Dairy Technology*, *63*(2), 209–215. http://doi.org/10.1111/j.1471-0307.2010.00567.x

Dahiya, R. S., & Speck, M. L. (1968). Hydrogen peroxide formation by lactobacilli and its effect on Staphylococcus aureus. *Journal of Dairy Science*, *51*(10), 1568–1572. http://doi.org/10.3168/jds.S0022-0302(68)87232-7

Dalu, J. M., & Feresu, S. B. (1996). Survival of Listeria monocytogenes in three Zimbabwean fermented milk products. *Journal of Food Protection*, *59*(4), 379–383. http://doi.org/10.4315/0362-028X-59.4.379

E.A., L. G. J. y J. (1992). Acid Adaptation Promotes Survival of Salmonella in Cheese. *Appl. Environ. Microbiol.*, *58*(6), 2075–2080.

Farnaud, S., & Evans, R. W. (2003). Lactoferrin - A multifunctional protein with antimicrobial properties. *Molecular Immunology*, *40*(7), 395–405. http://doi.org/10.1016/S0161-5890(03)00152-4

Feresu, S., & Nyati, H. (1990). Fate of pathogenic and non-pathogenic Escherichia coli strains in two fermented milk products. *The Journal of Applied Bacteriology*, *69*(6), 814–21. http://doi.org/10.1111/j.1365-2672.1990.tb01578.x

Gadaga TH, N. L. & A. M. (2004). THE OCCURRENCE, GROWTH AND CONTROL OF PATHOGENS IN AFRICAN FERMENTED FOODS. *African Journal of Food Agriculture Nutrition and Development*, *Vol. 4*(No. 1).

Government of New Zealand. (2001). Non-typhoid salmon, (May), 1–3.

Griffiths, M. W. (1986). Use of Milk Enzymes as Indices of Heat Treatment, *49*(9), 696–705.

Hammes, W. P., & Tichaczek, P. S. (1994). The potential of lactic acid bacteria for the production of safe and wholesome food. *Zeitschrift Für Lebensmittel-Untersuchung Und -Forschung*, *198*(3), 193–201. http://doi.org/10.1007/BF01192595

Herigstad, B., Hamilton, M., & Heersink, J. (2001). How to optimize the drop plate method for enumerating bacteria. *Journal of Microbiological Methods*, *44*(2), 121–129. http://doi.org/10.1016/S0167-7012(00)00241-4

HSE. (2013). Staphylococcus Aureus, (May), 1–4. Retrieved from http://www.hse.ie/eng/health/az/S/Staphylococcal-infections/

Hutkins, R. W., Nannan, N. L. (1993). pH Homeostasis in Lactic acid bacteria. *Department of Food Science and Technology*, *76*(8), 2354–2365.

Jans, C., Meile, L., Kaindi, D. W. M., Kogi-Makau, W., Lamuka, P., Renault, P., … Bonfoh, B. (2017). African fermented dairy products – Overview of predominant technologically important microorganisms focusing on African Streptococcus infantarius variants and potential future applications for enhanced food safety and security. *International Journal of Food Microbiology*. http://doi.org/10.1016/j.ijfoodmicro.2017.03.012

Jayarao, B. M., Donaldson, S. C., Straley, B. A., Sawant, A. A., Hegde, N. V., & Brown, J. L. (2006). A Survey of Foodborne Pathogens in Bulk Tank Milk and Raw Milk Consumption Among Farm Families in Pennsylvania. *Journal of Dairy Science*, *89*(7), 2451–2458. http://doi.org/10.3168/jds.S0022-0302(06)72318-9

Laulund, S., Wind, A., Derkx, P., & Zuliani, V. (2017). Regulatory and Safety Requirements for Food Cultures. *Microorganisms*, *5*(2), 28. http://doi.org/10.3390/microorganisms5020028

Lin, J., Lee, I. S., Frey, J., Slonczewski, J. L., & Foster, J. W. (1995). Comparative analysis of extreme acid survival in Salmonella typhimurium , Shigella flexneri , and Escherichia coli . Comparative Analysis of Extreme Acid Survival in Salmonella typhimurium , Shigella flexneri , and Escherichia coli, *177*(14), 4097–4104.

Lore, T. A., Mbugua, S. K., & Wangoh, J. (2005). Enumeration and identification of microflora in suusac, a Kenyan traditional fermented camel milk product. *LWT - Food Science and Technology*, *38*(2), 125–130. http://doi.org/10.1016/j.lwt.2004.05.008

Masschalck, B., & Michiels, C. W. (2003). Antimicrobial properties of lysozyme in relation to foodborne vegetative bacteria. *Critical Reviews in Microbiology*, *29*(3), 191–214. http://doi.org/10.1080/713610448

Motarjemi, Y. (2002). Impact of small scale fermentation technology on food safety in developing countries. *International Journal of Food Microbiology*, *75*, 213–229. http://doi.org/10.1016/S0168-1605(01)00709-7

Motarjemia, Y., & Kiifersteinb, F. K. (n.d.). Global estimation of food borne diseases, (1 997).

Moynihan, P. J., & Clarke, A. J. (2010). O-acetylation of peptidoglycan in gram-negative bacteria: Identification and characterization of peptidoglycan O-

acetyltransferase in Neisseria gonorrhoeae. *Journal of Biological Chemistry*, *285*(17), 13264–13273. http://doi.org/10.1074/jbc.M110.107086

Mufandaedza, J., Viljoen, B. C., Feresu, S. B., & Gadaga, T. H. (2006). Antimicrobial properties of lactic acid bacteria and yeast-LAB cultures isolated from traditional fermented milk against pathogenic Escherichia coli and Salmonella enteritidis strains. *International Journal of Food Microbiology*, *108*(1), 147–152. http://doi.org/10.1016/j.ijfoodmicro.2005.11.005

Nout, M. J. R., & Motarjemi, Y. (1997). Assessment of fermentation as a household technology for improving food safety: a joint FAO/VVHO workshop. *Food Control*, *8*(5/6), 221–226. http://doi.org/10.1016/S0956-7135(97)00021-2

Nyatoti, V. N., Mtero, S. S., & Rukure, G. (1997). Pathogenic Escherichia coli in traditional African weaning foods. *Food Control*, *8*(1), 51–54. http://doi.org/10.1016/S0956-7135(96)00068-0

Of, I., & Hydrogen, S. B. Y. (1969). INHIBITION OF PSEUDOMONAS SPECIES BY HYDROGEN, 13–18.

Ogwaro, B. A., Gibson, H., Whitehead, M., & Hill, D. J. (2002). Survival of Escherichia coli O157:H7 in traditional African yoghurt fermentation. *International Journal of Food Microbiology*, *79*(1–2), 105–112. http://doi.org/10.1016/S0168-1605(02)00184-8

Oliver, S. P., Boor, K. J., Murphy, S. C., & Murinda, S. E. (2009). Food Safety Hazards Associated with Consumption of Raw Milk. *Foodborne Pathogens and Disease*, *6*(7), 793–806. http://doi.org/10.1089/fpd.2009.0302

Oliver, S. P., Jayarao, B. M., & Almeida, R. A. (2005). Foodborne Pathogens in Milk and the Dairy Farm Environment: Food Safety and Public Health Implications. *Foodborne Pathogens and Disease*. http://doi.org/10.1089/fpd.2005.2.115

Ozturkoglu-Budak, S. (2018). Effect of different treatments on the stability of lysozyme, lactoferrin and β-lactoglobulin in donkey's milk. *International Journal of Dairy Technology*, *71*(1), 36–45. http://doi.org/10.1111/1471-0307.12380

Park, S., Worobo, R. W., & Durst, R. a. (1999). Escherichia coli O157:H7 as an emerging foodborne pathogen: a literature review. *Critical Reviews in Food Science and Nutrition*, *39*(6), 481–502. http://doi.org/10.1080/10408699991279259

Robinson, R. (2014). *Encyclopedia of food microbiology*. Academic press.

Rohrbach, B. W., Draughon, F. A., Davidson, P. M., & Oliver, S. P. (1992). Prevalence of Listeria monocytogenes, Campylobacter jejuni, Yersinia enterocolitica, and Salmonella in bulk tank milk: risk factors and risk of human exposure. *Journal of Food Protection*, *55*(2), 93–97.

Røssland, E., Borge, G. I. A., Langsrud, T., & Sørhaug, T. (2003). Inhibition of Bacillus cereus by strains of Lactobacillus and Lactococcus in milk. *International Journal of Food Microbiology*, *89*(2–3), 205–212. http://doi.org/10.1016/S0168-1605(03)00149-1

SÁNCHEZ, L., PEIRÓ, J. M., CASTILLO, H., PÉREZ, M. D., ENA, J. M., & CALVO, M. (1992). Kinetic Parameters for Denaturation of Bovine Milk Lactoferrin.

Journal of Food Science, *57*(4), 873–879. http://doi.org/10.1111/j.1365-2621.1992.tb14313.x

The council of the European communities. (1992). Council Directive (92/46/EEC). *Official Journal of the European Communities*, *46*(L 268/1), 1–32. http://doi.org/10.1039/ap9842100196

Tsegaye, M., & Ashenafi, M. (2005). Fate of Escherichia coli O157:H7 during the processing and storage of Ergo and Ayib, traditional Ethiopian dairy products. *International Journal of Food Microbiology*, *103*(1), 11–21. http://doi.org/10.1016/j.ijfoodmicro.2004.12.006

Wang, R.-F., Cao, W.-W., & Cerniglia, C. E. (1997). A universal protocol for PCR detection of 13 species of foodborne pathogens in foods. *Journal of Applied Microbiology*, *83*(6), 727–736. http://doi.org/10.1046/j.1365-2672.1997.00300.x

Wheeler, T. T., Hodgkinson, A. J., Prosser, C. G., & Davis, S. R. (2007). Immune components of colostrum and milk - A historical perspective. *Journal of Mammary Gland Biology and Neoplasia*, *12*(4), 237–247. http://doi.org/10.1007/s10911-007-9051-7

Whiting, R. C. (1993). Modeling bacterial survival in unfavorable environments. *Journal of Industrial Microbiology*, *12*(3–5), 240–246. http://doi.org/10.1007/BF01584196

World Health Organization. (1984). Role of Food Safety in Health Development.pdf. Retrieved from http://apps.who.int/iris/bitstream/10665/38709/1/WHO_TRS_705.pdf?ua=1

Wouters, J. T. M., Ayad, E. H. E., Hugenholtz, J., & Smit, G. (2002). Microbes from raw milk for fermented dairy products. *International Dairy Journal*, *12*(2–3), 91–109. http://doi.org/10.1016/S0958-6946(01)00151-0

Yeung, R. M. W., & Morris, J. (2001). Food safety risk. *British Food Journal*, *103*(3), 170–187. http://doi.org/10.1108/00070700110386728

APPENDIX

PRELIMINARY TEST CONSUMER QUESTIONNAIRE 1

Traditional fermented mabisi consumer questionnaire

[University of Zambia UNZA, Wageningen Universiteit]
Liv van de Ven

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

Date:

What is your gender?

- ☐ Male
- ☐ Female

What is your age?

- ☐ <20
- ☐ 20 – 29
- ☐ 30 – 39
- ☐ 40 – 49
- ☐ 50 – 65
- ☐ >65

What is your occupation?

- ☐ Student
- ☐ Unemployed
- ☐ 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

Place of birth:……

Current residence:……

Appendix 1

Please circle the word that best matches your response.

How often do you consume Mabisi: Daily / Weekly / Monthly / Yearly

Do you prefer: Traditional / Commercial

Do you think traditional Mabisi is safe: Yes / No

Which one do you think is safer: Traditional / Commercial

Where do you buy your traditional mabisi: ……

Where do you pay attention to when buying mabisi? .....

Where do you base your decision on most when buying mabisi? Rank according to importance to following five terms. 1 being most important, 5 being the least important to you.

Taste Availability Price Hygiene Visual appearance

Most important 1……..

2……..

3……..

4……..

Least important 5……..

Do you think mabisi producers/ farmers dilute the milk? Yes / No

When do you stop drinking mabisi: Too bitter / Too acidic / Too grainy / Never discard /other:……

Did you ever experience any illness after consumption of <u>fresh milk</u> (diarrhea, stomach ache, nauseous, vomiting, etc.) Yes / No

If yes, how often do you experience this: Always / Frequently / Sometimes / Once / other:…….

Did you ever experience any illness after consumption of <u>Mabisi</u> (diarrhea, stomach ache, nauseous, vomiting, etc.) Yes / No

If yes, how often do you experience this: Always / Frequently / Sometimes / Once / other:……..

Appendix 1

Did you ever had any physical inconvenience after consumption of Mabisi (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		Dizzyness		Slurred speech
	Bloody diarrhea		Loss of appetite		Muscle weakness		Death
	Vomiting		Fever		Difficulties in breathing		Other, namely……

Do you know someone who got ill from mabisi: Yes / No

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		Dizzyness		Slurred speech
	Bloody diarrhea		Loss of appetite		Muscle weakness		Death
	Vomiting		Fever		Difficulties in breathing		Other, namely……

Appendix 1

PRELIMINARY TEST CONSUMER QUESTIONNAIRE 2

Traditional fermented mabisi consumer questionnaire

[University of Zambia UNZA, Wageningen Universiteit]
Liv van de Ven

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

Date:

What is your gender?

- ☐ Male
- ☐ Female

What is your age?

- ☐ <20
- ☐ 20 – 29
- ☐ 30 – 39
- ☐ 40 – 49
- ☐ 50 – 65
- ☐ >65

What is your occupation?

- ☐ Student
- ☐ Unemployed
- ☐ 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

Place of birth:……

Current residence:……

Appendix 1

Please circle the word that best matches your response.

How often do you consume Mabisi: Daily / Weekly / Monthly / Yearly

Do you prefer: Traditional / Commercial

Do you think traditional Mabisi is safe: Yes / No

Which one do you think is safer: Traditional / Commercial

Where do you buy your traditional mabisi:……

Where do you pay attention to when buying mabisi? Tick all boxes that apply

O Hygiene of seller O Hygiene of selling place O Cleanness of buckets

O Place of selling O Rodents, insects O Other…….

Where do you base your decision on most when buying mabisi? Rank according to importance to following five terms. 1 being most important, 5 being the least important to you.

Taste Availability Price Hygiene Visual appearance

Most important 1……..

2……..

3……..

4……..

Least important 5……..

Do you think mabisi producers/ farmers dilute the milk? Yes / No

Do you drink the bought mabisi immediately: Yes / No

If no, how do you store your mabisi:……

When do you stop drinking mabisi: Too bitter / Too acidic / Too grainy / Never discard / other:……

Did you ever experience any illness after consumption of <u>fresh milk</u> (diarrhea, stomach ache, nauseous, vomiting, etc.) Yes / No

If yes, how often do you experience this: Always / Frequently / Sometimes / Once / other:……

Did you ever experience any illness after consumption of <u>Mabisi</u> (diarrhea, stomach ache, nauseous, vomiting, etc.) Yes / No

If yes, how often do you experience this: Always / Frequently / Sometimes / Once / other:……

Appendix 1

Did you ever had any physical inconvenience after consumption of Mabisi (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		Dizzyness		Slurred speech
	Bloody diarrhea		Loss of appetite		Muscle weakness		Death
	Vomiting		Fever		Difficulties in breathing		Other, namely……

Do you have any explanation why this happened?

	Bad hygiene of the cow		Unhygienic tools for storage of product
	Wrong fermentation		Unhygienic packaging of product
	Unhygienic tools for milking		Use of dirty/contaminated water for cleaning
	Unhygienic tools for fermentation		Use of dirty/contaminated water for diluting Mabisi
	Rodents or insects		Other, namely ……

Do you know someone who got ill from mabisi: Yes / No

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		Dizzyness		Slurred speech
	Bloody diarrhea		Loss of appetite		Muscle weakness		Death
	Vomiting		Fever		Difficulties in breathing		Other, namely……

Appendix 1

ASSOCIATION OF INTERVIEW QUESTIONS TO RESEARCH QUESTIONS

What is the consumer perception and awareness on perceived risk, food related hazards and hygienic practices associated with traditional fermented mabisi?

Are there differences in place of residence, making a division between urban, suburban(townships) and villagers? Are there differences in tribes? Are there differences between education

Questions involved: -Demographic data with all data linked

Where lays the consumers preference, when differentiating between commercial and traditional mabisi, and why? Is there question for more availability of the traditional drink?

Questions involved:
-1. How often do you consume mabisi?
-2. Do you prefer traditional /commercial ?
-3. Which one do you buy more often traditional /commercial?
Why do you buy this one more often?

Are consumers aware of risks involved with raw fermented milk?

Questions involved:
-4. Which one do you think is safer traditional/commercial ?
-5. Do you think traditional mabisi is safe?

Are consumers aware of risks when no good hygienic practices are assured? And how important is hygiene for them and what is their perception on good hygiene?

Questions involved:
-6. Where do you buy your traditional mabisi?
-12. Do you associate any food safety risks with traditional mabisi?
If yes, what risks do you associate with traditional mabisi?
-13. Does anything discourage you from buying traditional mabisi ?
If yes, what discourages you?
-14. How important is hygiene for you when buying mabisi?
-15. Do you think food hygiene is well practiced at the place you get your traditional mabisi from?
-16. Do you know what food poisoning is?

Are consumers aware of good storage practices concerning mabisi?

Questions involved:
-8. Do you drink the bought mabisi immediately?
-9. How do you store your mabisi?
-10.How long do you store it?
-11. When do you stop drinking mabisi?

Are people getting sick due to consumption of mabisi and is this due to food borne pathogens / lactose intolerance /other?

Questions involved:
-17. Do you know what lactose intolerance is?
-18. Did you ever experience any illness after consuming fresh milk?
If yes, how often do you experience this?
-19. Did you ever experience any illness after consuming mabisi?
If yes, how often do you experience this?
If yes, this experience was after consuming traditional/commercial?
If yes, what did you experience after getting sick from mabisi?
If yes, do you have any explanation why this happened?
-20. Do you know someone who got ill from consuming mabisi?
If yes, do you know which symptoms the person suffered from?

Appendix 1

TRADITIONAL MABISI CONSUMER QUESTIONNAIRE

Dear respondent,
Thank you for agreeing to take part in this survey on the traditional fermented milk, Mabisi. This survey is part of a bigger study on the safety and microbial stability against spoilage of traditional fermented beverages of Zambia. This project is a collaboration between the University of Zambia and Wageningen University of the Netherlands. This survey should only take 5-10 minutes to complete. Be assured that all answers you provide will be kept in strictest confidentiality.

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

Date: Name enumerator:

What is your gender?

- ☐ Male
- ☐ Female

What is your age?

- ☐ <20
- ☐ 20 – 29
- ☐ 30 – 39
- ☐ 40 – 49
- ☐ 50 – 65
- ☐ >65

What is your occupation?

- ☐ Student
- ☐ Unemployed
- ☐ 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:…….....

Residential area (township/suburb) inside Lusaka…….

Appendix 1

Please circle the word that best matches your response. For responses with the answer [other] please fill in answer on the dotted line. For open questions please fill in the answer on the dotted line.

1. How often do you consume mabisi: Daily / Weekly / Monthly / Yearly

2. Do you prefer: Traditional / Commercial

3. Which one do you buy more often: Traditional / Commercial
   Why do you buy this one more often: …….
   ….…….

4. Which one do you think is safer: Traditional / Commercial

5. Do you think **traditional** mabisi is safe: Yes / No

6. Where do you buy your **traditional** mabisi: Market / Farm / Family / Supermarket / Other……

7. Do you think mabisi producers dilute the milk? Yes / No / I don't know

8. Do you drink the mabisi you buy immediately? Yes / No / Sometimes

9. How do you store your mabisi: …….

10. How long do you store it: don't store / 1-2 days / 3-4 days / 5-7 days />7 days

11. When do you stop drinking mabisi: Too bitter / Too acidic / Too grainy / Watery / It's spoiled / Never discard / other:……

12. Do you think there is anything in **traditional** mabisi that can make you sick? Yes / No
If yes, what can make you sick?
……
……

13. Does anything discourage you from buying **traditional** mabisi ? Yes / No
If yes, what discourages you?
……
……

14. How important is hygiene for you when buying mabisi? On a scale from 1 to 10 circle your answer.

1	2	3	4	5	6	7	8	9	10
Not important at all				Neutral					extremely important

15. Do you think food hygiene is well practiced at the place you get your traditional mabisi from?

1	2	3	4	5	6	7	8	9	10
poorly practiced				Neutral					very properly practiced

16. Do you know what food poisoning is? Yes / No

17. Do you know what lactose intolerance is? Yes / No

18. Do you drink Mabisi against stomach upsets? Yes / No

19. Have you ever experienced any illness because of consuming **fresh milk**? Yes / No

If yes, how often do you experience this: Always / Frequently / Sometimes / Once / other:…….....

If yes, what kind of symptoms did you experience?.....

20. Have you ever experienced any illness because of consuming **Mabisi?** Yes / No

If yes, how often have you experienced this: Always / Frequently / Sometimes / Once / other:……

If yes, this experience was after consuming: Traditional / Commercial

If yes, what did you experience after getting sick from mabisi consumption? Please put the number of times you experience that feeling next to the box that describe the symptoms.

	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……

If yes, do you have any explanation why this happened?

……

……

21. Do you know someone who got ill from consuming mabisi: Yes / No

If yes, do you know which symptoms the person suffered from?

	I do not know which symptoms the person suffered from		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……

Thank You for taking the time to complete this questionnaire. We truly value the information you have provided. If you have any questions please let the enumerator know.

Appendix 1

TRADITIONAL FERMENTED MABISI PRODUCER QUESTIONNAIRE

[University of Zambia UNZA, Wageningen Universiteit]

Liv van de Ven

This survey should be combined with 2 samples of the producer:
- 1 of the fresh raw milk
- 1 of the finished mabisi sample

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

Date:…….. Name:…….....

Sales

1. Who do you produce mabisi for? (tick all that apply)?

- o my household/own home
- o Friends and family
- o For commercial use

If the answer in 1. "for commercial use", answer question 3:

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

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

3. How often do you produce your product?
- o Daily
- o A few times per week
- o Once per week
- o Monthly

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

- o <5 liters
- o 5-15 liters
- o 16-30 liters
- o 31-50 liters
- o >50 liters

5. How do you sell your product?

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

Process

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

Parameter	
Sieving of raw milk before use	Yes / no
If yes, what do you use for sieving?	
Do you use old mabisi as starter	Yes / no
Do you boil the milk before filling fermentation vessel	Yes / no
If yes for boiling, how long	……. minutes
Where do you store the fermenting mabisi?	o In the sun o In a water bath o In the shade o In a shed o In the house
How long is the fermentation time?	…….hours/days
Is the fermentation time longer in cold season? If yes please fill in	…….hours/days
What type of fermentation vessel do you use?	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:……
Draining of whey	Yes / no / sometimes
Sieving of end product	Yes / no / sometimes

Further comments to question 6:
……
……
……
……

7. How do you know that the fermentation process is completed?

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

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

8. How do you stop fermentation?

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

9. Do you add sugar to the mabisi?

- I do not add sugar to the product
- During the fermentation
- Before selling
- Other, namely ……

10. Do you use anything to standardize the process of making your product (tick all that apply)?

- No
- Measuring time
- Measuring temperature
- Measuring acidity (pH)
- Measuring viscosity
- Other, namely ……

Ingredients

11. Where do you get your raw milk from?

- Own cows
- From the farm
- Milk collection point
- Other, namely……

Do you bring your own collecting can/bottle? Yes / No
if no, in what is the milk collected?.....

12. How are the udders of the cow cleaned before milking?

- I have no idea
- They are not cleaned
- With tap water
- With boiled tap water
- By sucking calve
- With the cow's tail
- Other, namely……

13. Do you store the raw milk before making mabisi? Yes / No

If yes, 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 Pet o Repellant
o Other, namely ……

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

Discarding: yes/no

Defects: o I never discard anything o Smelly water
o Dirt in raw milk o Insects in raw materials
o Odd looking raw milk o Mould in fermentation vessels
o Smelly milk o Other, namely ……

Additional comments to question 14, in case further explanation is given:
……..……
……..……

Dilution

16. Do you ever dilute the raw milk or finished product with water? Yes / no

If yes, when do you add the water?

Addition: o Directly in raw milk o During fermentation

o To the finished mabisi o Other……

In case addition of water, do you boil this? Yes / no

Where is the source of water from?

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

17. Do you ever dilute the finished mabisi with new raw milk because the end product is not as preferred? Yes / No

If yes why do you dilute?

- o End product too thick
- o End product too thin
- o End product too grainy
- o End product too sour
- o Other, namely ……

If you add raw milk after, do you keep on fermenting? Yes / No

If yes for how long? …….hours/days

Hygiene

18. How do you clean your utensils/equipment (tick all that apply)?

- o I do not clean my utensils
- o Cold water
- o Hot water
- o Soap
- o Bleach/disinfectant
- o Other, namely ……

19. How do you clean your fermentation vessel (tick all that apply)?

- o I do not clean my tools
- o Cold water
- o Hot water
- o Soap with cold water
- o Soap with hot water
- o Bleach/disinfectant
- o Other, namely ……

20. Do you ever discard the finished product? Yes / No

Why?

- o I never discard the finished product
- o Insects in product
- o Too sour
- o Too bitter
- o Too much whey separation
- o Too grainy
- o Other, namely ……

Do you take other steps to ensure safety of your product? Yes / No
If yes, which?
……..……
……..……
……..……

Consumption

21. What are the storage conditions and shelf life you recommend to your customers?

Storage:	o Refrigerated/cooled	o Below room temperature
	o Room temperature	o Above room temperature
	o None	o Other, namely……

22. Shelf life at specified storage conditions:

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

23. How did you determine your shelf life?

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

24. Have you ever had any complaints/problems concerning your product? Yes / No

If yes, of what kind (Tick all that are applicable)?

- 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 ……

25. If yes, what do you believe was the source for the problems (Tick all that are applicable)?

- Wrong fermentation
- Contaminated raw milk
- Unhygienic utensils for fermentation
- Unhygienic utensils for storage of product
- Unhygienic packaging of product
- Use of dirty/contaminated water for cleaning
- Use of dirty/contaminated water for diluting mabisi
- Use of dirty/contaminated raw milk for diluting mabisi
- Other, namely ……

26. Have you ever heard of any other producer that had problems with his/her Mabisi? Yes / No

If yes, of what kind (Tick all that are applicable)?

- Product is spoiled
- Consumer got had discomforts after consumption
- Consumer got seriously ill after consumption
- Consumer died after consumption
- Other, namely ……

27. What are the characteristics of a spoiled product?

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

In case of own cows please answer these questions.
Could you describe the handling steps of milking the cow?

……...……
……...……
……...……
……...……

Are your cows tested on Mastitis? Yes / No
Which test do you use for this?

- California test
- Alcohol test
- Other, namely……

Do you still use this milk for mabisi? Yes / No

If a cow gets ill is it separated? Yes / No

When a cow gets medicine is it still milked for production? Yes / No

Hygiene perception

What do you believe is necessary to produce safe mabisi?

……
……
……
……

What do you think proper hygiene is?

……
……
……
……

What do you think are the risks involved with producing mabisi for the safety of the consumers?

……
……
……
……

To which step/what do you pay the most attention to while making mabisi?

……
……
……
……

Do you believe your way of making mabisi could be improved according to hygiene?

……
……
……
……

If you would upscale your process, would you change things? Or do you prefer your way of working now?

……
……
……
……

APPENDIX 1A

Table 12 Crosstable of highest level of education against knowledge on food poisoning and lactose intolerance. Data obtained from total of 172 subjects in SPSS. Significance of Fisher exact test is indicated with (*).

		What is your highest level of education?					
		No education	Primary	Secondary	Tertiary	University	Total
Knowledge food poisoning *	No	100,0%	92,3%	77,6%	60,6%	42,5%	69,2%
	Yes		7,7%	22,4%	39,4%	57,5%	30,8%
Knowledge lactose intolerance	No	100,0%	96,2%	97,0%	87,9%	87,5%	93,0%
	Yes		3,8%	3,0%	12,1%	12,5%	7,0%

Table 13 Selected cases that prefer traditional, purchase commercial. Answer of those cases on which product was bought more often.

		Frequency	Percent
Valid	Taste	1	3,4
	Availability	22	75,9
	Hygiene	1	3,4
	Safety	3	10,3
	Original	1	3,4
	no answer	1	3,4
	Total	29	100,0

Table 14 Mean and std. deviation on hygiene importance and hygiene practices of selling location for all subjects. Data analysed with SPSS.

Statistics

Compound/Choma		How important is hygiene for you when buying mabisi?	Do you think food hygiene is well practiced at places where mabisi is sold?
All Data	N Valid	172	172
	Mean	8.88	6.44
	Std. Deviation	1.958	2.839
	Minimum	0	0
	Maximum	10	10

Table 15 Compare groups between preference on safety of traditional mabisi in general. Data analysed with SPSS.

Do you prefer traditional or commercial mabisi?			Frequency	Percent	Valid Percent	Cumulative Percent
Traditional	Valid	Yes	114	88,4	88,4	88,4
		No	15	11,6	11,6	100,0
		Total	129	100,0	100,0	
Commercial	Valid	Yes	21	48,8	48,8	48,8
		No	22	51,2	51,2	100,0
		Total	43	100,0	100,0	

Table 16 Compare groups between preference on which product they perceive as safer. Data analysed with SPSS.

Do you prefer traditional or commercial mabisi?			Frequency	Percent	Valid Percent	Cumulative Percent
Traditional	Valid	Traditional	83	64,3	64,3	64,3
		Commercial	46	35,7	35,7	100,0
		Total	129	100,0	100,0	
Commercial	Valid	Traditional	5	11,6	11,6	11,6
		Commercial	38	88,4	88,4	100,0
		Total	43	100,0	100,0	

Table 17 Compare groups between preference. Counts and percentages on if subject relate illness to traditional product and if they are discouraged. Data analysed with SPSS.

Do you prefer traditional or commercial mabisi?			Illness related Frequency	Illness related Percent	Discouraged Frequency	Discouraged Percent
Traditional	Valid	No	82	63,6	64	49,6
		Yes	47	36,4	65	50,4
		Total	129	100,0	129	100,0
Commercial	Valid	No	21	48,8	19	44,2
		Yes	22	51,2	24	55,8
		Total	43	100,0	43	100,0

Table 18 Reasoning behind purchase comparing between purchase of traditional buyers and commercial buyers. Data obtained from SPSS frequencies.

Which one do you buy more often?			Frequency	Percent	Valid Percent	Cumulative Percent
Traditional	Valid	Taste	27	26,5	26,5	26,5
		Availability	2	2,0	2,0	28,4
		Price	12	11,8	11,8	40,2
		Nutrients	6	5,9	5,9	46,1
		Make own	24	23,5	23,5	69,6
		Safety	1	1,0	1,0	70,6
		Original	11	10,8	10,8	81,4
		No preservatives	5	4,9	4,9	86,3
		no answer	14	13,7	13,7	100,0
		Total	102	100,0	100,0	
Commercial	Valid	Taste	7	10,0	10,0	10,0
		Availability	34	48,6	48,6	58,6
		Nutrients	1	1,4	1,4	60,0
		Hygiene	6	8,6	8,6	68,6
		Safety	13	18,6	18,6	87,1
		Original	1	1,4	1,4	88,6
		Other	3	4,3	4,3	92,9
		no answer	5	7,1	7,1	100,0
		Total	70	100,0	100,0	

Table 19 Frequency of illness after consumption of raw milk. (split file on subjects that experienced illness after fresh milk). Data analysed in SPSS.

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	always	10	23,3	23,3	23,3
	frequently	3	7,0	7,0	30,2
	sometimes	24	55,8	55,8	86,0
	once	6	14,0	14,0	100,0
	Total	43	100,0	100,0	

Appendix 1

Table 20 Knowledge on lactose intolerance from subjects who always experience illness after consumption of fresh milk.

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	8	80,0	80,0	80,0
	Yes	2	20,0	20,0	100,0
	Total	10	100,0	100,0	

Table 21 Split file on subjects who experienced illness after consumption of mabisi and their safety and risk perception. Data analysed with SPSS.

Experienced illness after mabisi consumption		Traditional safe (count)	Traditional safe (%)	Traditional risk (count)	Traditional risk (%)	Discouraged (count)	Discouraged (%)
No	Yes	119	81,0	93	63,3	76	51,7
	No	28	19,0	54	36,7	71	48,3
	Total	147	100,0	147	100,0	147	100,0
Yes	Yes	16	64,0	10	40,0	7	28,0
	No	9	36,0	15	60,0	18	72,0
	Total	25	100,0	25	100,0	25	100,0

Table 22 Split file on subjects who experienced illness after consumption of mabisi and if they think traditional mabisi is safe. Data analysed with SPSS.

Experienced illness after mabisi consumption		Frequency	Percent
No	Traditional	79	53,7
	Commercial	68	46,3
	Total	147	100,0
Yes	Traditional	9	36,0
	Commercial	16	64,0
	Total	25	100,0

Table 23 Storage time of subjects who store their product after purchase in percentages and counts. File compared all cases, refrigeration temperature(n=89) and room temperature (n=43). Data analysed by SPSS.

		All cases (count)	All cases (%)	Fridge (count)	Fridge (%)	Room Temperature (count)	Room Temperature (%)
Valid	1-2 days	38	22,1	51	57,3	27	62,8
	3-4 days	89	51,7	23	25,8	15	34,9
	5-7 days	43	25,0	10	11,2		
	>7 days	2	1,2	5	5,6	1	2,3
	Total	172	100,0	89	100,0	43	100,0

Table 24 Reason behind discarding of the product, in percentages and counts. File compared all cases, refrigeration temperature(n=89) and room temperature (n=43). Data analysed with SPSS.

		All cases (count)	All cases (%)	Fridge (count)	Fridge (%)	Room Temperature (count)	Room Temperature (%)
Valid	Too bitter	61	35,5	32	36,0	16	37,2
	Too acidic	35	20,3	11	12,4	13	30,2
	Too grainy	2	1,2	2	2,2		
	Watery	20	11,6	10	11,2	7	16,3
	It's spoiled	10	5,8	8	9,0	1	2,3
	Never discard	22	12,8	12	13,5	2	4,7
	Other	22	12,8	14	15,7	4	9,3
	Total	172	100,0	89	100,0	43	100,0

Table 25 Purchase, preference and safer of traditional or commercial when file is split into place of residence. Counts and percentages obtained from SPSS.

		Purchase Percent %	Purchase count	Preference Percent %	Preference count	Safer Percent %	Safer count
Choma	Traditional	86,2	56	84,6	55	66,2	43
	Commercial	13,8	9	15,4	10	33,8	22
Lusaka	Traditional	45,2	33	68,5	50	39,7	29
	Commercial	54,8	40	31,5	23	60,3	44
Lusaka compound	Traditional	38,2	13	70,6	24	47,1	16
	Commercial	61,8	21	29,4	10	52,9	18

Table 26 Purchase location/access point, split file in residence (Choma, Lusaka, Lusaka compound). And consumption behaviour when splitting file in residence. Data analysed with SPSS.

Compound/Choma			Frequency	Percent
Choma	Valid	Market	12	18,5
		Farm	8	12,3
		Family	4	6,2
		Make own	35	53,8
		other	1	1,5
		Milk collection centre	5	7,7
		Total	65	100,0
Not Compound	Valid	Market	28	38,4
		Farm	22	30,1
		Family	15	20,5
		Make own	8	11,0
		Total	73	100,0
Compound	Valid	Market	15	44,1
		Farm	11	32,4
		Family	2	5,9
		Supermarket	3	8,8
		Make own	3	8,8
		Total	34	100,0

Compound/Choma			Frequency	Percent	Valid Percent	Cumulative Percent
Choma	Valid	Daily	29	44,6	44,6	44,6
		Weekly	27	41,5	41,5	86,2
		Monthly	8	12,3	12,3	98,5
		Yearly	1	1,5	1,5	100,0
		Total	65	100,0	100,0	
Not Compound	Valid	Daily	7	9,6	9,6	9,6
		Weekly	28	38,4	38,4	47,9
		Monthly	36	49,3	49,3	97,3
		Yearly	2	2,7	2,7	100,0
		Total	73	100,0	100,0	
Compound	Valid	Daily	5	14,7	14,7	14,7
		Weekly	20	58,8	58,8	73,5
		Monthly	6	17,6	17,6	91,2
		Yearly	3	8,8	8,8	100,0
		Total	34	100,0	100,0	

Table 27 Split file in place of residence (Choma, Lusaka, Compound). Purchase behaviour (traditional/commercial) percentages and counts. Data analysed with SPSS.

Compound/Choma			Frequency	Percent	Valid Percent	Cumulative Percent
Choma	Valid	Traditional	56	86,2	86,2	86,2
		Commercial	9	13,8	13,8	100,0
		Total	65	100,0	100,0	
Not Compound	Valid	Traditional	33	45,2	45,2	45,2
		Commercial	40	54,8	54,8	100,0
		Total	73	100,0	100,0	
Compound	Valid	Traditional	13	38,2	38,2	38,2
		Commercial	21	61,8	61,8	100,0
		Total	34	100,0	100,0	

Table 28 Split file in place of residence (Choma, Lusaka, Compound). Safety perception (traditional/commercial) percentages and counts. Data analysed with SPSS

Compound/Choma			Frequency	Percent	Valid Percent	Cumulative Percent
Choma	Valid	Traditional	43	66,2	66,2	66,2
		Commercial	22	33,8	33,8	100,0
		Total	65	100,0	100,0	
Not Compound	Valid	Traditional	29	39,7	39,7	39,7
		Commercial	44	60,3	60,3	100,0
		Total	73	100,0	100,0	
Compound	Valid	Traditional	16	47,1	47,1	47,1
		Commercial	18	52,9	52,9	100,0
		Total	34	100,0	100,0	

Appendix 1

Table 29 Split file in residence, preference, purchase. Percentages and counts given on reason for purchase behaviour. Data analysis obtained with SPSS.

Choma/Lusaka	Do you prefer traditional or commercial mabisi?	Which one do you buy more often?			Percent	Count
Lusaka	Traditional	Traditional	N=45	Taste	44,4	20
				Availability	2,2	1
				Price	8,9	4
				Nutrients	11,1	5
				Make own	13,3	6
				Safety	2,2	1
				Original	13,3	6
				No preservatives	4,4	2
		Commercial	N=28	Taste	3,6	1
				Availability	78,6	22
				Hygiene	3,6	1
				Safety	10,7	3
				Original	3,6	1
	Commercial	Commercial	N=31	Taste	12,9	4
				Availability	35,5	11
				Nutrients	3,2	1
				Hygiene	12,9	4
				Safety	32,3	10
				Other	3,2	1
Choma	Traditional	Traditional	N=41	Taste	17,1	7
				Availability	2,4	1
				Price	17,1	7
				Nutrients	2,4	1
				Make own	41,5	17
				Original	12,2	5
				No preservatives	7,3	3
	Commercial	Traditional	N=2	Price	50,0	1
				Make own	50,0	1
		Commercial	N=6	Taste	33,3	2
				Availability	16,7	1
				Hygiene	16,7	1
				Other	33,3	2

APPENDIX 1B

Traditional fermented mabisi producer questionnaire **Date of Q: 08-01-2018**

Producer 1: Suzan Nachigna (Siyokwa farm) **Tel: +0978936762**

Description: Farmer in Choma rural area, household production mainly

Sales
For own household/own home (main) and commercial use
Sold from home
2 times a week
production capacity each time between 5-15 L
sold directly from the fermentation vessel in containers brought by customers

Process

Raw milk is sieved before production by a tea strainer. No boiling of raw milk before fermentation.
Normally no backslopping, in cold times sometimes backslopping.
Fermented in a plastic bucket covered with the lid.
Fermentation takes place in the house for approximately 24 hours. In cold season without backslopping it takes 48 to 72 hours.
No draining of whey, no sieving of end product.
Checking of mabisi is ready is done by shaking (visual checking of thickening).
Fermentation stopped by cooling it (refrigerated).
Sugar only added for own consumption.
No standardizing of the process, only visual check if product is done.

Ingredients

Raw milk obtained from own cows.
Raw milk used for mabisi is not stored before but immediately set away in plastic bucket (different from milking can).

Milking

Cows are brought to the milking shed. There the udders are cleaned with warm water (borehole). Sucking of the calve is used to get the milking started. Cows are tested on mastitis at the Milk Collection Centre with the alcohol test. This milk is not used for mabisi anymore (milk arrived here normally not used for mabisi but only selling, so doubtful!) . If a cow is ill it gets separated but is still milked for production.

Hygiene
Nothing used to scare away insects and rodents.
Milk is discarded when yellow looking (odd looking milk). Not discarded when dirt of insects are found in the sieve.
Fermentation vessel is cleaned with warm water and soap after finishing mabisi, so not before.

Utensils and other equipment washed with water and soap.
Never discard the finished product.
Safety is ensured by putting the lid on the fermentation vessel the whole time.

Dilution

Milk never diluted with water.
Mabisi (end product) never diluted with new raw milk.

Consumption

No recommendations to consumers about shelf life and storage conditions (with comment that everyone always consumes within the same day).
Shelf life for home use not determined (with comment that only small quantities are produced, so no necessity).
Never had complaints/problems concerning the product. And never heard of other producers having problems.
Spoiled products is determined by a strange smell.

Hygiene perception

Necessary to produce a safe mabisi is mainly storage and handling. The product should be stored always with the lid on. The handling must be clean and careful.
Proper hygiene concerns the person and the environment. They should be clean.
There are no risks involved with production of mabisi, it is not common that anyone gets ill from mabisi.
Step that you have to pay the most attention to is storage.
To improve my process, the whole line from raw material to end product must be cleaner.
When upscaling I wouldn't change anything in my way of production.

Appendix 1

Traditional fermented mabisi producer questionnaire **Date of Q: 08-01-2018**

Producer 2: Grace Njanja **Tel: +0976308205**

Description: Farmer in Choma rural area, household production mainly

Sales
For own household/own home (main) and commercial use only on request
Sold from home
Daily small production
production capacity each time between <5 L
Sold directly from the fermentation vessel in containers brought by customers

Process
Raw milk is sieved before production by a tea strainer. No boiling of raw milk before fermentation (with the comment that this will give a different change).
No backslopping. But if they have a lot of milk left over they drain the under layer a couple of times when separation has occurred to remain most of the creamy/fat part. Which results in a high quality mabisi.
Fermented in a plastic bucket covered with the lid.
Fermentation takes place in the house for approximately 24 hours. In cold season without backslopping they also ferment for 24 hours, with the comment that the mabisi then is less thick.
No sieving of end product.
Checking of mabisi is ready is done by shaking (visual checking of stickiness) and tasting (should be bitter and taste the sweetness of fresh milk).
Fermentation is not stopped, they let it ferment further until everything is used (but small batch so quickly used).
No sugar added.
Standardized is always on room temperature and always 24 hours of fermentation.

Ingredients
Raw milk obtained from own cows. Milking in milk can.
Raw milk used for mabisi is not stored before but immediately set away in plastic bucket (different from milking can).

Milking
Cows are brought to the milking shed. There the udders are cleaned with cold water (well). The udder should be massaged and lubricated.

Cows are tested on mastitis at the Milk Collection Centre with the alcohol test. If they are tested positive they will do the same test at home or the California test. This milk is not used for mabisi anymore (milk arrived here normally not used for mabisi but only selling, so doubtful!) . If a cow is ill it is not separated and not milked for production.

Appendix 1

Hygiene
Cat is used to scare away insects and rodents. Mainly closing with the lid is ensuring hygiene.
Milk is discarded when dung is found. Cows next time are driven to a dry place and knees are put together (dung mainly found in rainy season).
Fermentation vessel is cleaned with warm water and soap before use of vessel.
Utensils and other equipment washed with water and soap.
Discarded the finished product because it was too bitter.
Safety is ensured by healthy cows, milking when calves are young and avoiding of overfermentation.

Dilution
Milk never diluted with water.
Mabisi (end product) never diluted with new raw milk. But with the comment that if she would add milk she would boil it before addition.

Consumption
Recommendations to consumers about shelf life and storage conditions are store at room temperature. In hot season for maximum of three days and cold season maximum of 5 days. This is determined by visual appearance and experience.
Shelf life for home use not determined (with comment that only small quantities are produced, so no necessity).
Never had complaints/problems concerning the product. Heard of other producers having problems, product was spoiled, consumers had discomfort after consumption (diarrhoea). Spoiled products is determined by a different taste and lighter appearance and grainy separation.

Hygiene perception
Necessary to produce a safe mabisi is after collection of milk immediately start fermentation at room temperature. Always ensure that there is a separation occurring.
Proper hygiene concerns the milk. The milk itself should be clean and milk shouldn’t have any particles.
There are no risks involved with production of mabisi.
Step that you have to pay the most attention to is sieving of the milk. Proper milking techniques, the cleaning of the udder/utensils should be proper. The udder should be massaged and lubricated.
Improvement and upscaling plans could be paddock for the animals, standard milking parlour or robots, improve animal breed, standardize process and draining in bulk containers to obtain high quality mabisi only.

Appendix 1

Traditional fermented mabisi producer questionnaire **Date of Q: 08-01-2018**

Producer 3: Roreen Mucjhanga Mudenda **Tel: +0974424680**

Description: Farmer in Choma rural area, household production mainly

Sales
For own household/own home (main), friends and family and commercial use (only on request).
Sold from home
Daily production
Production capacity each time between 16-30 L
Sold directly from the fermentation vessel in containers brought by customers

Process
Raw milk is sieved before production by a tea strainer.
No boiling of raw milk before fermentation.
No backslopping.
Fermented in a plastic bucket covered with the lid.
Fermentation takes place in the house for approximately 24 hours. In cold season without backslopping they ferment for 48-72 hours.
No sieving of end product. No draining of end product. Checking of mabisi is ready is done by visual checking of thickness and smelling of aroma change.
Fermentation is not stopped, they let it ferment further until everything is used. If this takes to long they add new raw milk and ferment it again
No sugar added, only for own consumption.
Standardized is always on room temperature because they put the bucket in a water bath at room temperature.

Ingredients
Raw milk obtained from own cows. Milking in milk can.
Raw milk used for mabisi is not stored before but immediately set away in plastic bucket (different from milking can).

Milking
Cows are brought to the milking shed. There the udders are cleaned with cold water (pipeline). Milk collected in milking can. Cows are tested on mastitis at the Milk Collection Centre with the alcohol test. This milk is not used for mabisi anymore, only for calves. If a cow is ill it is separated from the herd and not milked for production when medicated.

Hygiene
Nothing is used to scare away insects and rodents. Mainly closing with the lid is ensuring hygiene.
Never discarded raw milk before.
Fermentation vessel is cleaned with cold water and soap before use of vessel.
Utensils and other equipment washed with cold water.
Discarded the finished product because it was not thick enough.
No other steps are taken to ensure safety.

Dilution
Milk never diluted with water.
Mabisi (end product) never diluted with new raw milk.

Consumption
Recommendations to consumers about shelf life and storage conditions are store at room temperature. For maximum of two to three days. This is determined by own experience.
Shelf life for home use not determined (with comment that only small quantities are produced, so no necessity).
Never had complaints/problems concerning the product. Never heard of other producers having problems.
Spoiled products is determined by other factor. No milk should ferment less than one day.

Hygiene perception
Necessary to produce a safe mabisi is that the container must be clean and the milk must be clean.
Proper hygiene concerns well cleaned utensils, milking should be clean and the person should be clean.
There are no risks involved with production of mabisi.
Step that you have to pay the most attention to is cleanness of the container. Improvement and upscaling plans are changing the whole structure of the farm, change breed, milking equipment and place. More standardization.

Appendix 1

Traditional fermented mabisi producer questionnaire **Date of Q: 09-01-2018**

TRANSLATED

Producer 4: Grace Mutazhe Farm **Tel: +260979305750**

Description: Farmer in Choma rural area, household production and selling

Sales
For own household/own home and through a seller from the market in Choma.
At local markets
Once a week production
Production capacity each time between 5-15 L
Sold directly from the fermentation vessel in containers brought by customers

Process
Raw milk is sieved before production by a four corner sieve.
No boiling of raw milk before fermentation.
No backslopping.
Fermented in a plastic bucket covered with the lid.
Fermentation takes place in the house for approximately 48 hours. In cold season without backslopping they ferment for 72 hours.
No sieving of end product. No draining of end product.
Checking of mabisi is ready is done by visual checking of thickness and graininess.
Fermentation is not stopped, they let it ferment further until everything is used or sold.
No sugar added.
Standardized is always on room temperature.

Ingredients
Raw milk obtained from own cows. Milking in milk can.
Raw milk used for mabisi is not stored before but immediately set away in plastic bucket (different from milking can).

Milking
Cows are brought to the milking place. There the udders are cleaned with cold water (in hot season) or hot water (in cold season). Udders are massaged with udder crème, before milking.
Milk collected in milking can. Cows are tested on mastitis with California test. This milk is not used for mabisi anymore. If a cow is ill it is separated from the herd and not milked for production when medicated.

Hygiene
Pet and repellent is used to scare away insects and rodents.
Never discarded raw milk before.
Fermentation vessel is cleaned with hot water, bleach and soap before use of vessel.
Utensils and other equipment washed with soap with hot water.
Never discarded the finished product.
Other steps taken to ensure safety are covering with the lid, milk must be clean and fermentation vessel has to be clean.

Appendix 1

Dilution
Milk never diluted with water.
Mabisi (end product) never diluted with new raw milk.

Consumption
Recommendations to consumers about shelf life and storage conditions are store somewhere in the kitchen. For maximum of two days (hot season) or three to four days in cold season. This is determined by own experience.
Never had complaints/problems concerning the product. Never heard of other producers having problems.
Spoiled products is determined by bitterness and watery appearance.

Hygiene perception
Necessary to produce a safe mabisi is that the milk must be clean; sieved and disease clean. Proper hygiene concerns proper milk, clean bucket, clean environment and room temperature. There are no risks involved with production of mabisi its always safe. Step that you have to pay the most attention to is closing the bucket with the lid. Improvement and upscaling plans are a specific room for production of mabisi. Feeding the animals with concentrates, clean water, milking parlour and more cows.

Appendix 1

Traditional fermented mabisi producer questionnaire **Date of Q: 09-01-2018**

TRANSLATED

Producer 4: Jane Pangwa **Tel: +260979150728**

Description: Farmer in Choma rural area, household production and selling

Sales
For commercial use
From home
Few times a week production
Production capacity each time between 16-30 L
Sold directly from the fermentation vessel in containers brought by customers

Process
Raw milk is sieved before production by a squared strainer.
No boiling of raw milk before fermentation.
No backslopping.
Fermented in a milk can covered with the lid. With the comment that the process is faster in the milk can because of the material.
Fermentation takes place in the house for less than 24 hours. In cold season without backslopping they ferment for 72 hours.
No sieving of end product. No draining of end product.
Checking of mabisi is ready is done by visual checking if cream top is present.
Fermentation is not stopped, they let it ferment further until everything is sold.
No sugar added. Standardized is always same bucket at the same place in the house.

Ingredients
Raw milk obtained from own cows. Milking in milk can.
Raw milk used for mabisi is not stored before but immediately set away in plastic bucket (different from milking can).

Milking
Cows are brought to the milking place. There the udders are cleaned with warm water and soap.Milk collected in milking can. Cows are tested on mastitis by someone else only if suspicion. This milk is not used for mabisi anymore. If a cow is ill it is not separated from the herd and not milked for production when medicated.

Hygiene
Repellent is used to scare away insects and rodents. Only discarded raw milk if mastitis was present.
Fermentation vessel is cleaned with cold water and disinfectant before use of vessel.
Utensils and other equipment washed with cold water.
Discarded the finished product when there was dirt inside or flies.
Other steps taken to ensure safety are covering with the lid,

Appendix 1

Dilution
Milk never diluted with water.
Mabisi (end product) never diluted with new raw milk.

Consumption
Recommendations to consumers about shelf life and storage conditions are not given because try to sell everything the same day.
Had complaints/problems about adulteration. She believes the problem was the fermentation style, her product was not diluted but separated. Never heard of other producers having problems.
Spoiled products is determined by insects, such as maggots, inside.

Hygiene perception
Necessary to produce a safe mabisi is that the product is covered with a lid and that the fermentation vessel is clean.
Proper hygiene concerns washing with soap.
There are no risks involved for consumers when producing mabisi.
Step that you have to pay the most attention to is closing the bucket with the lid.
Improvement and upscaling plans are a specific room for production of mabisi. More equipment to sell more raw milk because it is safer than mabisi. Raw milk contains more nutrients.

Appendix 1

Traditional fermented mabisi producer questionnaire **Date of Q: 09-01-2018**

Producer 6: Crecious Munsakq **Tel: +260954404569**

Description: Mtandalike Milking Collection Centre (Choma)

Sales
For commercial use
From Milk Collection Centre
Monthly only when it goes sour
Production capacity each time between >50 L
Sold directly from the fermentation vessel in containers brought by customers

Process
Raw milk is sieved before production by farmer if correct
No boiling of raw milk before fermentation.
No backslopping.
Fermented in a milk can covered with the lid. With the comment that the milk can no separation occurs, but in plastic container it does so you have to stir.
Fermentation takes place in the MCC for 48-72 hours. In cold season without backslopping they ferment for 120-168 hours.
No sieving of end product. No draining of end product.
Checking of mabisi is ready is done by visual checking if it gets thick and the taste has to become sour.
Fermentation is not stopped, they let it ferment further until everything is sold even if it takes days.
No sugar added.
Standardized is fresh milk is tested on everything. Only if it doesn't passes the sour test (alcohol test) it is used for mabisi. So only rejected milk on freshness.

Ingredients
Raw milk obtained from farmers brought in a milking can.
Raw milk used for mabisi is sometimes stored before in a cooled bulk tank.

Milking

-

Rejected milk is used. In rainy season reject all sour milk so no mabisi is made. In rainy season it's a bigger chance that cows get mastitis. Due to more dirt. Hot season the sleeping place of cows dryer.

Hygiene
Nothing is used to scare away insects and rodents.
Only discarded raw milk if mastitis was present or dirt was found.
Fermentation vessel is cleaned with cold water and disinfectant before use of vessel.
Utensils and other equipment washed with cold water and bleach.
Never discarded the finished product.
Other steps taken to ensure safety are covering with the lid.

Dilution
Milk never diluted with water.
Mabisi (end product) never diluted with new raw milk.

Consumption
No recommendations to consumers about shelf life and storage conditions.
Never had complaints/problems concerning product.
Heard of other producers having problems with separation of water because of plastic container. Or fermentation in sunlight which went wrong.
Spoiled products is determined by bitter taste and mould on top.

Hygiene perception
Necessary to produce a safe mabisi is that the product is covered with a lid. Proper hygiene starts from farmers, they have to wash their hands and equipment and then everything must be closed when making mabisi.
There are no risks involved for consumers when producing mabisi.
Step that you have to pay the most attention to is closing the bucket with the lid.
Improvement and upscaling plans painting the room (mould in room), mosquito net over the container, equalize the floor and repellent for insects. Own cows, more containers and cans and standard production line.

Appendix 1

Traditional fermented mabisi producer questionnaire **Date of Q: 08-01-2018**

Producer 6: Ms. A. Muchindu (Office manager)/ Milk collection centre Choma

Description: The Choma district dairy co-operative union and milk collection centre

700 farmers. 5 primary cooperatives (sell on their behave and collect). They produce yoghurt (stirred, starter culture, with flavours), lacto (pasteurized milk for mabisi) and traditional mabisi.

Sales
For commercial use
From Milk Collection Centre + another market point + other MCC
Production 2 times a week
Production capacity each time minimum of 200 L
Sold from 40 L cans if fermented into this vessels or plastic buckets (not same as fermentation vessel). Customers bring their own containers or buy it within a plastic bag.

Process
Raw milk is sieved before production by farmer if correct, before mabisi production MCC sieves the milk manually through filter paper.
No boiling of raw milk before fermentation.
No backslopping.
Fermented in a plastic container covered with the lid (40 L) or in 200 L drums. With the comment that the taste in plastic containers is better than the can.
Fermentation takes place in the MCC storage room for 48 hours (room T that day was 35 degrees). In cold season without backslopping they ferment for <120 hours (depending on the temperature).
No sieving of end product. No draining of end product. Only stirring of end product.
Checking of mabisi is ready is done by visual checking if it gets thick (put in dipping stick, when nothing is dripping it is done) and the taste has to become sour.
Fermentation is stopped by transferring it to another container which is cooled.
No sugar added.
Standardized are the test they preform before the milk is collected from the farmers.
If they have a small quantity of milk for mabisi production the containers are not washed and the process is faster.

Ingredients
Raw milk obtained from farmers brought in a milking can.
Raw milk used for mabisi is sometimes stored before in a cooled bulk tank. (Fluctuating temperature below zero and way higher).
Comment Jersey cow milk used, for mabisi Friesian cow is more watery.

Milking
Farmers get cans or containers mainly from Parmalat. The milking parlour has to be certificated in health and personal hygiene. Most farmers wash the udders with luke warm water and use milking salve. No milk is taken when mastitis (California test) is found or when its medicated (goes to calves). In rainy season a different conformation test is used to be reassured.

They test on alkakine (mastitis, adulteration with hydroxide) and freshness (pH, alchohol 75%). They use the lactoscan, alcohol test, California test, pH test.

In the receiving books the test results from each farmer are written down. When the milk is rejected they write down the name, amount and reason. Mainly hygiene problems (more DAZ trainings).

In rainy season the rejection is mainly due to mastitis followed by freshness. In dry season rejection is mainly due to adulteration (adding of water).

Hygiene
Nothing is used to scare away insects and rodents.
Only discarded raw milk if test results were positive for mastitis.
Disinfection points in MCC, on the ground. Disinfection for bulk tank every time after emptying with water, biofilm solution, water again.
Fermentation vessel is cleaned with cold water and disinfectant before use of vessel. (pipeline)
Utensils and other equipment washed with cold water and bleach.
Discarded the finished product, fermenting for over a week. No user friendly taste and smelly.
Other steps taken to ensure safety is no storage longer than a week.

Dilution
Milk never diluted with water.
Mabisi (end product) diluted with new raw milk when its to bitter. They keep on fermenting for at least 12 hours more after addition of new raw milk.

Consumption
No recommendations to consumers about shelf life and storage conditions.
Had complaints/problems concerning product about bitterness and thickness.
Did not hear of other producers having problems.
Spoiled products is determined by bitter taste, presence of bubbles or foam and presence of whey separation.

Hygiene perception
Necessary to produce a safe mabisi is clean milk which is disease free.
Proper hygiene starts from standards ZAB, GMP and GHP.
There are no risks involved for consumers when producing mabisi.
Step that you have to pay the most attention to is storage.
Improvement and upscaling plans are tiling the processing hall, marketing strategies and better equipment.

Appendix 1

Traditional fermented mabisi producer questionnaire **Date of Q: 29-01-2018**

Producer 8: Mbangu Farm **Tel: +260979400438**

Description: Farmer in Lusaka rural area

Sales
For commercial use (only on order, or through seller)
Brought at home and through sellers
3 times a week production
Production capacity 60 L min and 120 L maximum in a week
Sold from pre-packaged bottles

Process
Raw milk is sieved before production by a squared sieve.
No boiling of raw milk before fermentation.
No backslopping.
Fermented in a plastic bucket or in a milk can (depends on the order) covered with the lid.
Fermentation takes place in a cool place (summer) or at room T (rainy) for less than 48 hours.
No sieving of end product, ruins the product by breaking up the structure. Draining of end product, remove watery part. (too hot collect more water (water is down), water on top is taken with a cup).
Checking of mabisi is ready is done by visual checking of thickness, it should be compact and a bit loosely.
Fermentation is not stopped, it goes immediately to the customer.
No sugar added, only for own consumption.
Standardized is weather environment determines the process, thus controls the fermentation place.

Ingredients
Raw milk obtained from own cows. Milking in milk can.
Raw milk used for mabisi is not stored before but immediately set away in plastic bucket (different from milking can).

Milking
Cow importance; water supply must be clean, rest and grazing place should be clean. Mostly mastitis when there are no regular milkers. More milk left in the udder. Every milker has their own set of cows, buckets, places in the fridge. (collective responsibility, if something goes wrong)

Cows are brought to the milking parlour. Which is cleaned before and after milking. There the udders are cleaned with cold water (borehole). Udders are salved with crème. Milk collected in milking can. Cows are tested on mastitis with alcohol test and once more at home with boiling clotting test. This milk is not used for mabisi anymore, only for calves. If a cow is ill it is separated from the herd and not milked for production when medicated.

Appendix 1

Hygiene
Repellent is used to scare away insects and rodents (when milk is not there). Mainly closing with the lid is ensuring hygiene.
Discarded raw milk before because of dirt, odd looking, smelly, insects, sour, mastitis, power outage, soap left in can.
Fermentation vessel is cleaned with cold water and soap, rinsed with hot water and dried in the sun, stored in the fridge until used.
Utensils and other equipment washed with cold water and soap and rinsed with hot water.
Discarded the finished product because it had insects, too sour, too bitter, too grainy, transport time was too long.
Other steps are taken to ensure safety are check quality of mabisi, broken mabisi is reduced quality so brought back to the fridge to settle.

Dilution
Milk never diluted with water.
Mabisi (end product) diluted with new raw milk if too little is present for the order. Not fermented afterwards.

Consumption
Recommendations to small consumers only about shelf life and storage conditions are store refrigerated. For maximum of one to two days. This is determined by own experience (tasting, visual).
Had complaints/problems concerning the product about consistency (too watery). Thinks its because wrong fermentation. Never heard of other producers having problems.
Spoiled products is determined by bitter taste, presence of whey separation, number of days fermented.

Hygiene perception
Necessary to produce a safe mabisi is good animal health, hygiene from beginning to end, time scheduling (preparation, delivery, transport, fermentation).
Proper hygiene concerns handling of the milk and personnel should be clean. Process from cow to fridge, the time to leave the farm to selling time.
There are risks involved with production of mabisi due to hygiene.
Step that you have to pay the most attention to is cleanness of the container.
Improvement and upscaling plans are enhancing milk parlour, cooling system for milking (direct cooling), equipment. Would change if market wants change.

Traditional fermented mabisi producer questionnaire **Date of Q: 18-01-2018**

Producer 9: Mischeck Muzungu Kakonde **Tel: +260972563394**

Description: Farmer in Lusaka rural area

Sales
For my own household and friends and family
From home
Monthly production
Production capacity each time 5-15 L
Sold from fermentation vessel to customer (package is vessel)

Process
Raw milk is sieved before production by a tea strainer
No boiling of raw milk before fermentation.
No backslopping.
Fermented in a plastic bucket covered with the lid.
Fermentation takes place in a cool place at room T for less than 24 hours. In cold season <48 hours.
No sieving of end product. No draining of end product.
Checking of mabisi is ready is done by visual checking of sedimentation process, water is coming up. Shake to check the consistency and taste for sour taste. (thickness is done by dipping a cup).
Fermentation is not stopped, it keeps on fermenting, has to be consumed within a week stored at a cool place.
No sugar added, only for own consumption.
No standardization.

Ingredients
Raw milk obtained from own cows. Milking in milk can.
Raw milk used for mabisi is stored before at the farm at room T and in town in the fridge.

Milking
Cows are milked in the grassland. First let the calve suck to stimulate the milk production. Then udders are cleaned by the tail. Milk collected in milking can. Cows are cleaned by vet through a dipping tank. Tested for mastitis by alcohol test. This milk is not used for mabisi anymore, only for calves. If a cow is ill it is separated from the herd and not milked for production when medicated.

Hygiene
Nothing is used to scare away insects and rodents.
Discarded raw milk before because of insects and bad taste.
Fermentation vessel is cleaned with cold water and soap, rinsed with hot water.
Utensils and other equipment washed with hot water and soap. Discarded the finished product because it had insects or dirty container.
No other steps are taken to ensure safety are check quality of mabisi.

Dilution
Milk never diluted with water.
Mabisi (end product) diluted with new raw milk if end product is too sour. Fermented further for 3 more hours.

Consumption
Recommendations to consumers about shelf life and storage conditions are store refrigerated. For maximum of 2-7 days. This is determined by own experience (tasting, visual).
Never had complaints/problems concerning the product. Heard of other producers having problems with adulteration.
Spoiled products is determined by colour (brownish) and off standard taste (watery).

Hygiene perception
Necessary to produce a safe mabisi is milking of the cow should be as clean as possible plus the milking environment. Segregate milking from sleeping place of the animals.
Proper hygiene concerns being able to milk the cow in a clean environment + proper health and medication of the cow + personal hygiene of handlers.
There are risks involved with production of mabisi due to hygiene. Mabisi has more health benefits than negative ones.
Step that you have to pay the most attention to is buckets should be clean.
Improvement and upscaling plans are ensuring weekly cleaning of animal placing, animals must be brushed and cleaned, machines also cleaned before milking (no bacteria), packaging must be nicely, different sizes of packaging, label plus branding, milking machines, different fermentation vessels, improve of milking.
Preserve milk so consumers can make their own mabisi.

APPENDIX 2

Table 30 Nanodrop results first run DNA extraction samples. Producers mabisi samples M1-9, Producers raw milk samples M1R-M7R and mabisi Trader samples T1-5.

RUN 1	SAMPLE ID	NUCLEIC ACID CONC. (NG/µL)	A260	A280	260/280	260/230
	M1	7.3	0.147	0.089	1.66	0.15
	M2	19.5	0.391	0.246	1.59	0.26
	M3	18.6	0.373	0.225	1.66	0.19
	M4	19.5	0.39	0.265	1.47	0.34
	M5	13.4	0.268	0.163	1.65	0.25
	M6	12.3	0.245	0.139	1.76	0.22
	M7	10.1	0.201	0.117	1.72	0.21
	M8	13.7	0.274	0.179	1.53	0.29
	M9	31.2	0.624	0.443	1.41	0.36
	M1R	9.4	0.188	0.12	1.57	0.18
	M2R	48.9	0.978	0.774	1.26	0.66
	M3R	11.1	0.222	0.147	1.51	0.23
	M4R	11.1	0.223	0.144	1.54	0.22
	M5R	11.4	0.229	0.146	1.57	0.20
	M6R	9.2	0.185	0.113	1.64	0.18
	M7R	14.6	0.292	0.178	1.64	0.25
	T1	29.3	0.587	0.43	1.37	0.44
	T2	30.7	0.615	0.439	1.4	0.44
	T3	26.5	0.53	0.373	1.42	0.43
	T4	24.6	0.493	0.343	1.44	0.37
	T5	8.4	0.168	0.115	1.47	0.18
	T6	23.1	0.461	0.328	1.41	0.41

Table 31 Nanodrop results second run DNA extraction samples. Producers mabisi samples M1-9, Producers raw milk samples M1R-M7R and mabisi Trader samples T1-5.

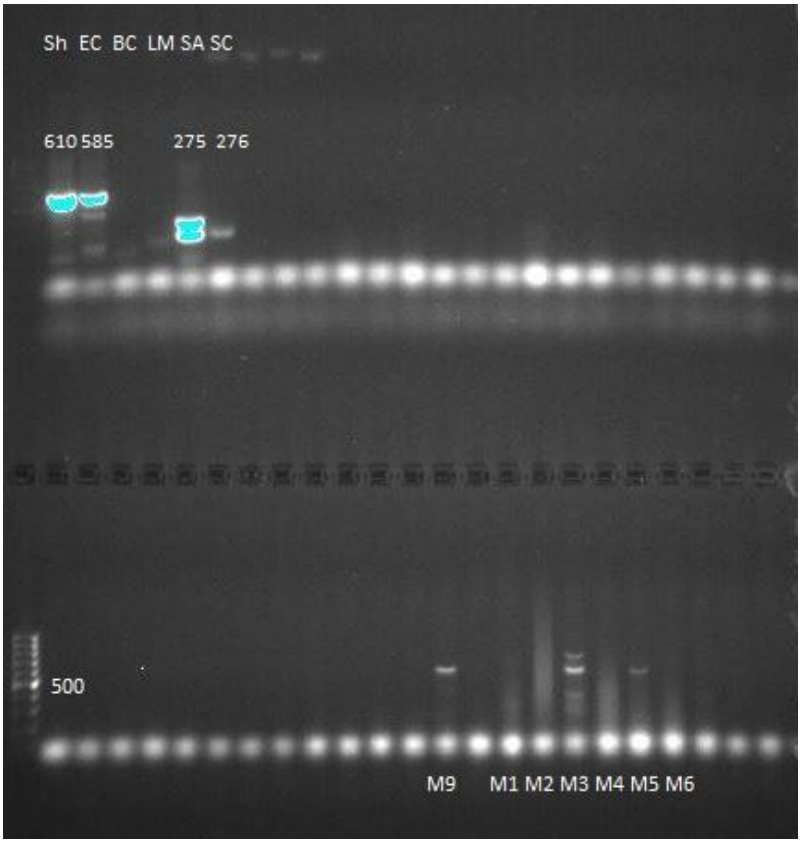
RUN 2	SAMPLE ID	NUCLEIC ACID CONC. (NG/µL)	A260	A280	260/280	260/230
	M1	158.8	3.177	2.081	1.53	1.19
	M2	309.2	6.185	3.913	1.58	1.26
	M3	135.2	2.703	1.925	1.40	1.00
	M4	411.1	8.222	5.145	1.60	1.40
	M5	201.1	4.021	3.679	1.09	0.77
	M6	287.2	5.744	3.652	1.57	1.23
	M7	87.2	1.745	1.533	1.14	0.77
	M8	56.6	1.133	0.949	1.19	0.78
	M9	37	0.739	0.642	1.15	0.69
	M1R	79.9	1.598	1.325	1.21	0.65
	M2R	67.4	1.347	1.2	1.12	0.49
	M3R	77.3	1.547	1.387	1.12	0.59
	M4R	56.4	1.128	0.915	1.23	0.55
	M5R	91.6	1.833	1.675	1.09	0.62
	M6R	74.3	1.486	1.294	1.15	0.77
	M7R	142.8	2.855	2.206	1.29	0.96
	T1	271.3	5.425	3.696	1.47	1.10
	T2	221	4.419	3.249	1.36	1.11
	T3	314	6.28	4.396	1.43	1.28
	T4	39.9	0.798	0.685	1.16	0.59
	T5	38.8	0.777	0.622	1.25	0.61
	T6	102.3	0.739	1.737	0.43	0.65

Table 32 Nanodrop results third run DNA extraction samples. Producers mabisi samples M1-9, Producers raw milk samples M1R-M7R and mabisi Trader samples T1-5.

RUN 3	SAMPLE ID	NUCLEIC ACID CONC. (NG/µL)	A260	A280	260/280	260/230
	M1	154.1	3.081	2.04	1.51	0.97
	M2	69.1	1.382	1.156	1.2	0.43
	M3	195.9	3.918	3.165	1.24	0.41
	M4	54.8	1.096	0.874	1.25	0.41
	M5	153	3.06	2.572	1.19	0.48
	M6	234.8	4.696	3.363	1.4	1.00
	M7	106.6	2.132	1.768	1.21	0.53
	M8	74.8	1.497	1.079	1.39	0.65
	M9	64.7	1.294	0.966	1.34	0.52
	M1R	52.1	1.041	0.816	1.28	0.53
	M2R	55.8	1.117	0.799	1.4	0.62
	M3R	136	2.719	2.13	1.28	0.78
	M4R	93	1.861	1.519	1.23	0.70
	M5R	93	1.86	1.511	1.23	0.65
	M6R	71.2	1.424	1.143	1.25	0.52
	M7R	43.3	0.867	0.66	1.31	0.46
	T1	122.7	2.453	1.995	1.23	0.59
	T2	200	4.001	3.063	1.31	0.72
	T3	106.2	2.124	1.564	1.36	0.53
	T4	421.1	8.423	5.777	1.46	1.13
	T5	62.3	1.245	0.962	1.29	0.59
	T6	112.6	2.252	1.848	1.22	0.59

Appendix 2

Table 33 Agarose gel with results for corrosponding samples. Ladders (bp) upper left and right and down left and right side. Positive controls for *Shigella spp.* (SH), *E. coli* (EC), *Bacillus cereus* (BC), *L. monocytogenes* (LM), *Salmonella spp.* (SA) and *S. aureus* (SC).



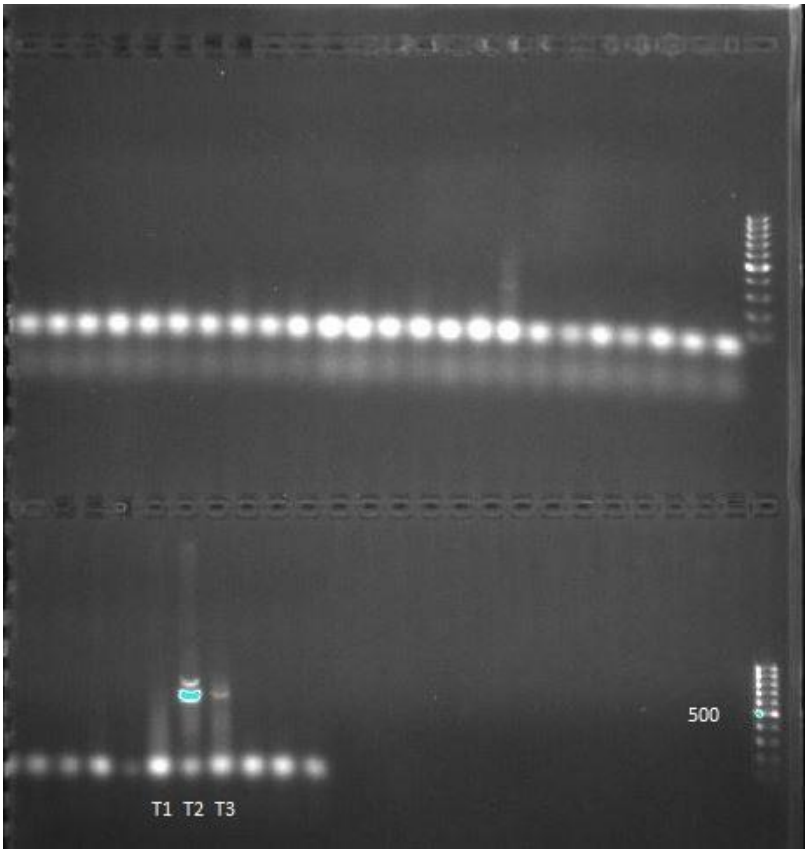


Table 34 Inoculation rates experiments performed in the Netherlands. Over time is survival of pathogen during fermentation over time. Static 1 is invasion during fermentation. Static 2 is survival during storage.

	Pathogen	**AVERAGE LOG CFU/mL + St. DEV**
Over time	*E. coli*	5.77 ± 0.55
	S. aureus	4.98 ± 0.064
Static 1	*L. monocytogenes*	6.12 ± 0.077
	Salmonella spp.	5.82 ± 0.040
	B. cereus	4.99 ± 0.119
Static 2	*E. coli*	5.38
	S. aureus	4.97
	L. monocytogenes	5.07
	Salmonella spp.	5.23
	B. cereus	5.05

Table 35 Pathogen growth detected in all samples (n=14), producers (n=9) and traders (n=5). Division made between producers and traders.

	All Samples (n=14)	**Producers (n=9)**	**Traders (n=5)**
Bacillus *cereus*	0/14	0/9	0/5
Shigella	0/14	0/9	0/5
Staphylococcus aureus	10/14	5/9	5/5
Indole producer	10/14	7/9	3/5
Salmonella ssp.	2/14	2/9	0/5

Table 36 Invasion of pathogen (LM/SA/BC/EC/SC) in raw milk during fermentation of 48 hours at 25 degrees. pH is obtained by sampling in the beginning and at the end of fermentation.

	0 (h)	**48 (h)**
L. monocytogenes	6.67 ± 0.001	4.28 ± 0.151
Salmonella spp.	6.67 ± 0.001	4.17 ± 0.072
B. cereus	6.67 ± 0.001	4.16 ± 0.011
E. coli	6.67 ± 0.001	4.02 ± 0.004
S. aureus	6.67 ± 0.001	4.05 ± 0.011
Control	6.67 ± 0.001	4.22 ± 0.000

Table 37 Invasion of pathogen (LM/SA/BC/EC/SC) after fermentation of 48 hours at 25 degrees. pH is obtained by sampling after 2 and 4 days of storage at 4 degrees.

	End fermentation	**Storage**	
	48 h (pH)	**96 h (2 day)**	**144 h (4 days)**
L. monocytogenes	4,22 ± 0,00	4,21 ± 0,01	4,15 ± 0,01
Salmonella spp.	4,22 ± 0,00	4,19 ± 0,02	4,13 ± 0,01
B. cereus	4,22 ± 0,00	4,24 ± 0,02	4,18 ± 0,02
E. coli	4,22 ± 0,00	4,20 ± 0,01	4,16 ± 0,01
S. aureus	4,22 ± 0,00	4,16 ± 0,01	4,13 ± 0,03
Control	4,22 ± 0,00	4,14 ± 0,00	4,14 ± 0,00