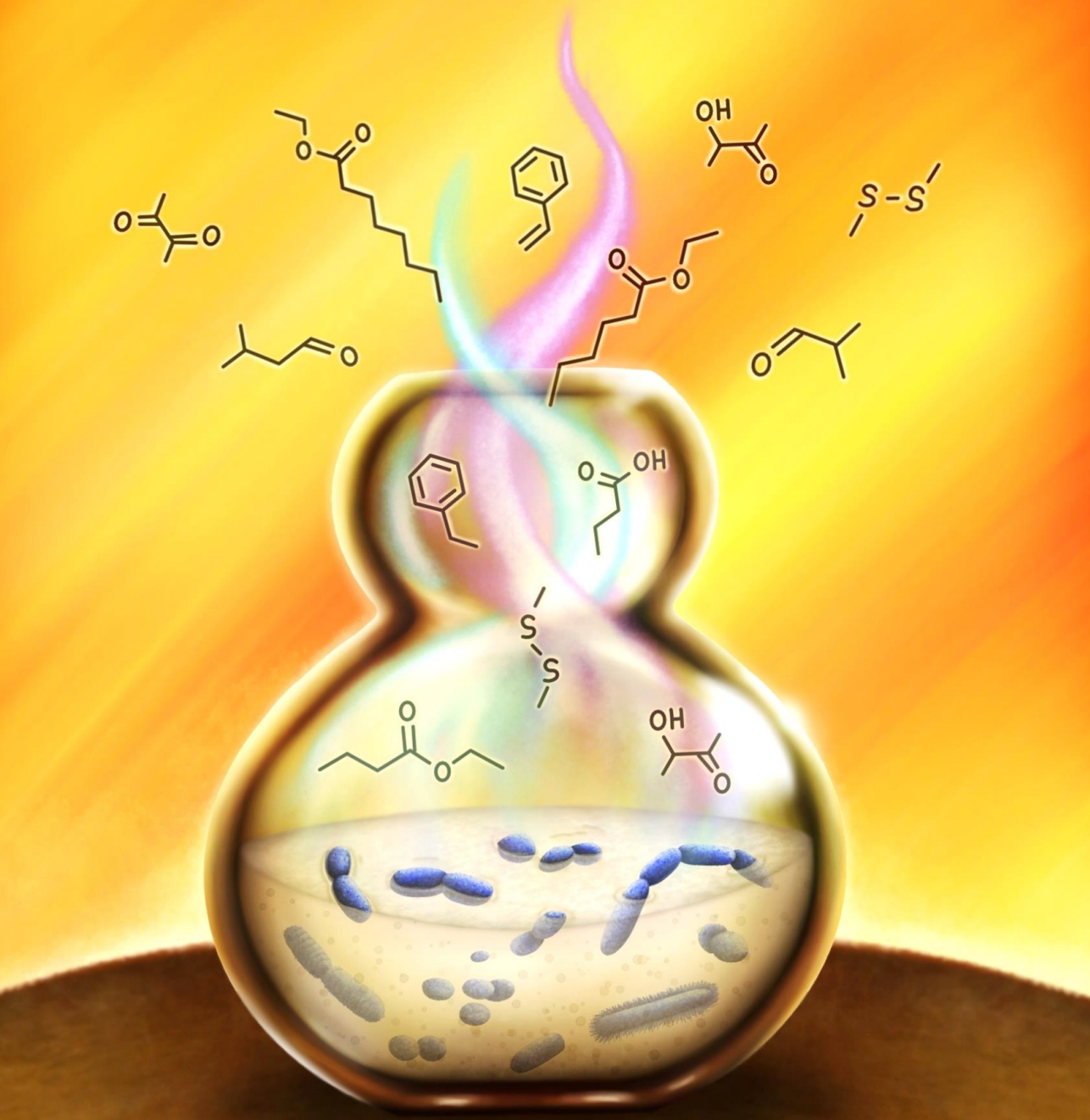
Traditional mabisi production,

exploring the characteristic properties of a spontaneously fermented milk product for process optimization.



Thelma W. Sikombe

Propositions

1) Each batch of the traditionally fermented mabisi is a historically unique mixture.
   (This thesis)

2) Mabisi consumers prefer a spontaneously fermented version of mabisi.
   (This thesis)

3) The lack of the public understanding of science, derails scientific progress.

4) There is no objectivity in the academic peer review process.

5) Social media's influence on food safety risk communication is a double-edged sword.

6) Remote working stifles brainstorming.

Propositions belonging to the thesis, entitled

Traditional mabisi production: Exploring the characteristic properties of a spontaneously fermented dairy product for process optimization

Thelma W Sikombe

Wageningen, 25 November, 2024

Traditional mabisi production

Exploring the characteristic properties of a spontaneously fermented dairy product for process optimization

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This research was conducted under the auspices of VLAG Graduate School (Biobased, Biomolecular, Chemical, Food, and Nutrition sciences)

Traditional mabisi production

Exploring the characteristic properties of a spontaneously fermented dairy product for process optimization

Thelma W. Sikombe

Thesis
submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr C. Kroeze,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Monday 25 November 2024
at 10.30 a.m in the Omnia Auditorium

Thelma W. Sikombe
Traditional mabisi production: Exploring the characteristic properties of a spontaneously fermented dairy product for process optimization
185 pages

PhD Thesis, Wageningen University, Wageningen, the Netherlands (2024) With references, with summary in English

ISBN: 9789465103068
DOI: 10.18174/675965

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CHAPTER

General introduction

Thelma W. Sikombe

1.1 Traditional fermented foods and their significance

Fermentation is a metabolic process in which microorganisms, such as bacteria, yeasts, and/or molds, break down sugars and complex organic compounds into various metabolic products (Caplice & Fitzgerald, 1999; Feng et al., 2018; Terefe, 2016). Traditional food fermentation is one of the oldest processing techniques known globally after drying. Its application to food preservation dates back to 6000 BC (Bintsis & Papademas, 2022; Fox, 2011; Jaya Shankar, 2023; Kurmann, 1992). As an ancient processing technique, fermentation is known to preserve the quality of various foods by transforming the raw materials of highly perishable items into shelf-stable products through the production of organic acids, alcohols, bacteriocins, and other compounds that inhibit the growth of spoilage and pathogenic microorganisms (e.g., *Staphylococcus aureus*, *Campylobacter jejuni*, *Listeria monocytogenes,* coliforms, *Escherichia coli,* and *Vibrio cholera*) (Fox, 2011; Schoustra et al., 2022). Thus, the metabolic compounds of fermentation play a crucial role in enhancing food safety. Apart from preservation, fermentation has been applied to food raw materials to enhance their nutritional value by breaking down complex food molecules into simpler, palatable, and more digestible forms. Additionally, fermentation can improve the organoleptic qualities of various animal and plant food raw materials through transformation into a wide range of products with different textures and unique flavors.

Fermented foods and beverages have a long history in virtually all human civilizations and are still an important part of the diets of many people worldwide. Knowledge of fermented food production was traditionally passed down through generations within families and communities (Marshall & Mejia, 2011). In the developed world, the fermentation processes for many products have been refined for large-scale commercial production. For instance, the manufacturing of cheese, yogurt, “sauerkraut” (i.e., fermented cabbage), soy sauce, and

fermented sausages have undergone significant advancements to meet the demands of mass production (Bintsis & Papademas, 2022; Tamang et al., 2020). These refinements include the use of defined or undefined mixed starter cultures, optimized fermentation parameters, and the application of modern technology to ensure batch-to-batch consistency (Leroy & De Vuyst, 2004; Mannaa et al., 2021). As a result, many fermented products can be produced in large quantities with consistent quality to meet consumer expectations.

Fermented foods cover a vast spectrum of products with an estimated 5,000 different foods globally (Suzzi & Corsetti, 2020). Regional variations in, for example, raw materials, fermenting microorganisms, fermentation times, processing methods, and environmental conditions distinguish these fermented foods (Mannaa et al., 2021). Every culture has its range of fermented foods that form part of the diet and is typically dependent on locally available raw materials as well as various other factors such as social, cultural, religious, and economic considerations (Hesseltine & Wang, 1980). The various raw materials include but are not limited to cereals, milk, legumes, tubers, fruits, vegetables, and meat resulting in a wide variety of products such as cheese, yogurt, bread, beer, wine, miso, and sausages, among others (Cuvas-Limon et al., 2021; Suzzi & Corsetti, 2020). Milk is one of the most ubiquitous raw materials used for making a wide variety of fermented milk products worldwide (Bintsis & Papademas, 2022). On the African continent, traditional fermented dairy products such amasi, nunu, sethemi, kivuguto, mursik, and masai are widely produced and consumed, besides fermented cereals (munkoyo, mahewu, and akpan) and brewed alcoholic beverages (kachasu, chibuku, and others) (Franz et al., 2014; Phiri et al., 2019; Pswarayi & Gänzle, 2019; Sacca et al., 2012; Sanya et al., 2023).

1.2 Fermentation for sustainable food systems

Recognized for its ability to prevent losses of highly perishable food raw materials and to support food security, fermentation serves as a vital, simple, low-cost food preservation technique. This is particularly important in low-income settings in the developing world where fermentation is the bedrock of small-scale food processing enterprises that are crucial to rural development. In areas with limited energy resources, fermentation allows communities to process and preserve perishable foods without requiring specialized equipment or electricity, making it a sustainable and accessible solution for improving food availability and reducing waste. Fermented foods are key in ensuring food and nutrition security by providing affordable nutritious foods for vulnerable communities (Balasubramanian et al., 2024). Moreover, an increasing body of evidence has emerged over the past decade, highlighting the functional and health benefits of fermented foods (Szutowska, 2020; Wastyk et al., 2021). By embracing traditional food fermentation methods, we can draw upon lessons from the past to support the development of resilient and innovative food systems for the future (Knorr et al., 2020). The food system is at the core of many of the UN sustainable development goals (SDGs). As the world grapples with food insecurity and challenged food systems, indigenous knowledge plays a significant role in achieving sustainable development and providing clean food production technologies. Therefore, traditional fermentation in food processing is a powerful means of enhancing food security. Traditional fermentation can empower local food processors with a sustainable stream of income, thereby improving the livelihoods and household nutrition security. As a universal technology, traditional fermentation offers opportunities for small-scale processors to achieve a number of the SDGs such as poverty (SDG 1) and hunger (SDG 2) reduction, good health and well-being (SDG 3), and improved livelihoods (SDG8) (Akinsemolu, 2018).

1.3 The growing consumer interest in fermented foods

Despite the role of fermentation in extending the shelf life of foods, fermented foods are increasingly becoming a health trend. There has been a rising interest in fermented foods among consumers in the last few years. The renewed interest is almost entirely based on consumer health and wellness. Gut health, in particular, posits fermented foods as a highly valued functional food on most consumers' shopping lists. In addition, the popularity and significance of fermented foods among consumers are due to their unique sensory appeal, and enhanced nutritional profile.

There is a significantly growing body of evidence supporting the health benefits of fermented products. Research on the gut microbiota and host physiology has linked the consumption of traditional fermented foods to diverse health benefits (Rul et al., 2022; Tamang, Shin, et al., 2016). For instance, fermented milk products like yogurt and kefir, have been shown to reduce health risks associated with type 2 diabetes, osteoporosis, and decreased brain activity, among others (Dimidi et al., 2019). The reported health benefits of fermented products have coincided with increased consumer demand and a growing market. This trend is likely to drive changes in the production and supply chain structure of traditional fermented products, such as the need to increase production volumes and improve packaging. While production scale-up is important, preserving the traditional aspects and cultural significance of traditional foods remains key. This would involve the use of indigenous methods of production already used in traditional processing.

In recent years, the food industry has significantly evolved, enhancing its ability to provide consumers with a diverse array of safe and superior quality products. This evolution has fostered increased consumer awareness regarding product safety and quality standards. Despite consumers' general perspective of fermented foods as safe, the growing awareness of food

safety requirements and the rising expectations for high-quality products are challenging producers to meet these demands. Consumers are increasingly attuned to food safety requirements despite their perception of fermented foods as healthy and nutritious.

1.4 Fermented dairy products

Milk is readily available wherever pastoral agriculture and animal husbandry are practiced, and milk from various mammals including cows, sheep, goats, and ewes has been used historically in fermentation (Jans et al., 2017). However, cow milk is the major type of milk used in fermentation processes worldwide (Bintsis & Papademas, 2022). Milk is a highly nutritious food, providing proteins, fats, vitamins, and minerals essential for human health. In many African countries, milk and fermented milk products are inherent in the traditional food supply chain and are highly recommended for a healthy diet. However, the rich nutrient profile makes milk also an ideal growth environment for invasion by various foodborne microorganisms, many of which may be spoilage or pathogenic agents rendering it highly perishable (Quigley et al., 2013). While the dairy sector in developed nations is largely industrialized, and characterized by routine application of pasteurization technologies, the dairy sector in developing countries, especially Africa, is less industrialized and dominated by many smallholder dairy farmers and processors. This is hampered by the warm climatic conditions, compounded by the lack of cold storage facilities, particularly in rural areas where electrical power supply is limited. Most of the milk in these areas is sold to the milk collection centres (MCC) and the remainder is further allowed to ferment naturally into sour milk and later sold in the local markets. The fermentation of milk is a natural process driven by the metabolism and enzymatic activities of the autochthonous microbes largely dominated by Lactic Acid Bacteria (LAB). Over the years, the fermentation production process has evolved, and sometimes a technique known as 'backslopping' is incorporated, where an aliquot from a

previously successful batch is used to initiate the fermentation of a new batch (Holzapfel, 2002).

Zambia boasts a diverse range of local fermented foods comparable to global products like yogurt, wine and beer. In addition, there are a few non-alcoholic traditional products like mabisi, munkoyo and chibwantu (Schoustra et al., 2013). Whereas munkoyo and chibwantu are cereal-based products, mabisi is milk-based. The latter is a popular fermented dairy product that has been consumed for centuries. It is characterized by an acidic taste with a varied but typically thick consistency determined by the production method. Its wide consumption spans regions, social classes, ages, and gender. It is generally accepted as a healthy and nutritious beverage, often taken alone as a snack or in combination with other foods (Moonga et al., 2019). Occasionally, it is used as a weaning food for infants. It is also a common belief among the locals that mabisi consumption can alleviate incidences of diarrhea in infants and young children, potentially due to the ability of the mabisi microbial community to regulate the gut microbiota and positively impact the immune system. For instance, a randomized study of natural yogurt supplemented with *Lacticaseibacillus casei* demonstrated reduced incidences of diarrheal episodes in young children (Pedone et al., 2000). Besides, mabisi has the potential for diet diversification to help improve people's nutritional status, particularly vulnerable groups. With the recent dietary suggestions to include diverse food components on our plates, regular consumption of mabisi is now recommended in the Zambian national food-based dietary guidelines (Chilton et al., 2015; GRZ, 2021).

Production of mabisi is a small-scale, household-level undertaking dominated by women processors. It is normally produced for sale in the local community market to supplement household incomes. Mabisi is considered a product of high nutritional and economic significance to these communities due to the low production costs and the high nutritional value

typical of fermented dairy products (Materia et al., 2021). Household-level enterprises are often synonymous with low production volumes, unpredictable and questionable quality, low incomes, and limited use of appropriate production and storage facilities (Holzapfel, 1997; Motarjemi, 2002). They present less consistent quality because the specific fermenting microbes vary, depending on many factors such as the raw milk used, environmental conditions, the producer, and the containers used in the fermentation process.

Historically, mabisi production was common among pastoral and cattle-rearing rural communities of the Southern, Western, and Central provinces of Zambia. However, over time and with the impact of urbanization, it is no longer an exclusive product for those communities but widely spread across the country (Moonga, 2019). Some brands of commercial variants of mabisi are available on the market and these have been produced from the simple defined sour milk starter cultures. Despite the widespread consumption of traditional mabisi, its production capacity falls short of consumer demand, as access is limited by geography and only those in places where raw milk is available have easy access.

1.4.1 The current production practices and uses of mabisi

A country-wide study of the traditional practices in mabisi production reported seven distinct mabisi production methods and their microbial communities across Zambia (Moonga et al., 2019). The production methods include tonga, barotse, backslopping, creamy, cooked, illa, and thick-tonga mabisi. Variations among the production methods include differences in fermentation time, application of back-slopping technique, use of heat-treated milk as raw material, agitation during the fermentation combined with removal of the formed butter, and the alternate removal of whey and addition of fresh milk during the production process. A schematic diagram showing the different production processes for four mabisi variants is outlined in Figure 1 (Moonga et al., 2019). The production of tonga-type mabisi is the most

practiced and preferred method by different ethnic groups across the country. Despite the variations in production methods, indigenous knowledge is crucial to the various techniques in mabisi production, and traditional approaches passed down through generations are still followed. The art of mabisi production has probably evolved over the years, though it is generally produced by the spontaneous fermentation of raw bovine milk at ambient temperatures for 24 - 48 hours.

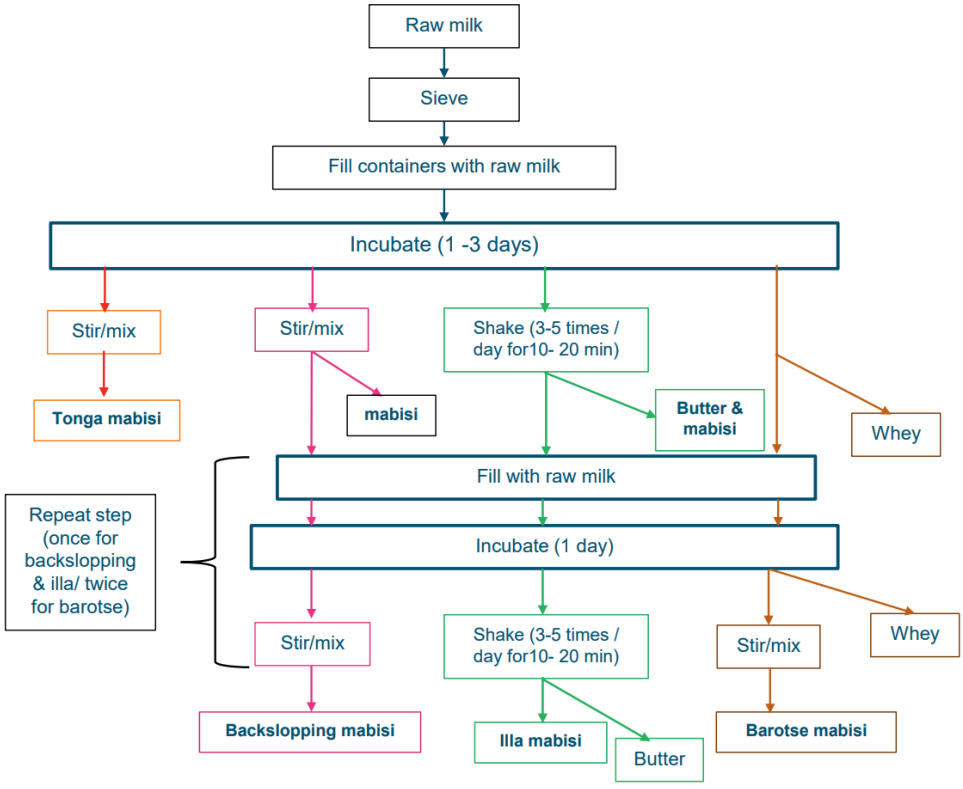

Fig. 1. A schematic presentation for the production of four types of mabisi namely tonga, backslopping, illa, and barotse-type mabisi, based on the work by Moonga and colleagues (2019).

Additionally, mabisi is a versatile product consumed in various ways, including as an everyday snack or during special gatherings, as an addition to popular dishes (e.g nshima, rice, pumpkin, and samp), and as an ingredient in baking and cooking (Fig. 2). Mabisi is also a well-known product in neighboring Namibia where it is produced similarly to the Zambian barotse-type mabisi. Its production involves the removal of whey and the addition of fresh milk, typically repeated three to four times or until whey production is substantially reduced (Misihairabgwi & Cheikhyoussef, 2017).

Fig. 2. A typical serving of pumpkins accompanied by mabisi.

1.4.2 The microbial ecology of mabisi

Although the fermentation of milk in Africa has a long history, knowledge of the microbial community of these fermented milk products is quite recent (Franz et al., 2014; Jans et al.,

2017). Amplicon sequencing of the hypervariable (V3–V4) regions of the 16S rRNA gene has revealed the taxonomy and diversity of the microbial community in mabisi production, which is largely driven by Lactic Acid Bacteria (LAB), although Acetic Acid Bacteria (AAB) and yeasts are also strongly involved albeit in lower abundances. The complex and diverse bacterial community is dominated by microorganisms from the two phyla *Pseudomonadota* and *Bacillota*. The genera *Lactococcus* sp. and former *Lactobacillus* sp. are particularly the most abundant species. Other bacterial species such as *Streptococcus, Kluyvera, Klebsiella, Enterobacter, Citrobacter, Buttiauxella, Aeromonas,* and *Acinetobacter* have also been reported (Moonga et al., 2020). Variations occur depending on the production method, geographical location, mabisi producer, the fermentation temperature, pH of the final product and type of container. The fermentation process may take between 24 – 48 hours during the hot season and may extend to 3 - 5 days during the cold seasons of the year when temperatures drop to below 20^{o}C. Different types of containers are used for mabisi production ranging from calabashes (the dried fruit of the plant *Lagenaria leucantha*) and clay pots to plastic and metal containers, depending on availability and the producer's preference (Fig. 3).

The effect of temperature, representing average ambient temperatures around the country, on the rate of fermentation and product characteristics has been reported in mabisi (Moonga et al., 2021). Higher temperatures typically accelerate the fermentation process compared to lower temperatures, resulting in products with a relatively lighter consistency due to increased syneresis. The composition of the microbial community also varies with temperature. Lower temperatures (20 - 25°C) are largely dominated by *Lactococcu*s sp., while higher fermentation temperatures demonstrate an increased presence of *Lactobacillus* sp. (Moonga et al., 2021). However, the impact of temperature variations on microbial community composition is reported to be specific to the production method.

Products of spontaneous fermentation usually show a large degree of variation in quality and organoleptic properties. The variations, which may range from flavor, texture, and appearance, are associated with the microbial community composition of the raw material, the environment in which the fermentation is conducted, as well as the utensils used in the process (Moonga et al., 2019; Zulu et al., 1997). Therefore, the application of process control measures and producer hygiene practices are crucial in assuring a good quality final product. Most consumers have attested to preferring the natural and rich organoleptic properties of the traditional mabisi compared to the commercially available alternative products in retail stores and supermarkets.

Fig. 3. Different types of containers used in the production of traditional mabisi; a) Plastic containers, b) metallic containers, c) calabashes, and d) Earthen pots

It is therefore a promising opportunity for current small-scale producers to make traditional mabisi available in an appealing manner to satisfy and exceed the expectations of consumers. Moreover, the need for sustainable food production has become more critical than ever. This would require that traditional mabisi producers scale up their production capacity and increase the availability of traditional products on the market. This is important in meeting the market's demands and ensuring that the product complies with regulatory requirements for food safety and quality. However, like with most traditional foods in Africa, the challenge of larger-scale production is the lack of optimization of production, which in turn yields an inconsistent final product (Mattiello et al., 2018).

1.5 Challenges with traditional fermentation

Traditional fermentation, with or without backslopping, is initiated by autochthonous microbes. It is prone to contamination and inconsistent results as the undefined and natural communities of microorganisms that initiate and drive the fermentation vary depending on many factors already mentioned and are genetically highly diverse (Tamang, Watanabe, et al., 2016). While traditional mabisi is widely appreciated as a healthy product with the potential to improve the nutritional status of people in vulnerable communities and better the livelihoods of its mostly small-scale producers, the use of unstandardized milk usually yields inconsistent and unpredictable final products regarding quality and microbiological safety. Microbiological safety is supported largely by the hostile environment created as a result of the low pH due to the presence of organic acids. Therefore, the rate of acidification is crucial for preventing the growth of unwanted microbes, maintaining food safety, and ensuring product quality. Moreover, other metabolic compounds such as hydrogen peroxide, and a broad spectrum of potent antimicrobials also play an important role in ensuring safety (De Vuyst & Leroy, 2007; Ouwehand & Vesterlund, 2004). The use of unpasteurized milk in traditional fermentation continues to raise public health concerns due to the lack of established quality standards for acceptable raw milk. As consumers nowadays are more informed about the quality and safety of the food on the market, producers must strictly uphold good manufacturing practices to instil confidence among the consumers. This includes eliminating potential contamination from unwanted microorganisms that constitute a significant microbial safety risk. Alternatively, pasteurization of milk could effectively eliminate microbial contamination from the raw milk (Holsinger et al., 1997). However, using pasteurized milk will necessitate the application of starter cultures to kick-start the fermentation.

Starter cultures offer the advantage of a more controlled production that is possible at different levels of scale. It also assures consistency in product quality and organoleptic properties, which is largely absent in the current production approaches. Alternatively, native mabisi starter cultures can be applied to initiate controlled microbial and metabolic activities while improving the fermentation process and yielding a more predictable final product (Mannaa et al., 2021). Several studies have shown that using native starter cultures could preserve the characteristics that define the authenticity and uniqueness of traditional foods (Palavecino Prpich et al., 2021; Sánchez Mainar et al., 2017; Vogel et al., 2011). Preserving these unique traditional fermented foods helps protect the hidden value of local biodiversity (Galimberti et al., 2021). Similarly, the application of starter cultures would offer better opportunities for production scale-up, providing the basis for the diversification of mabisi variants on the market and maximizing profits for producers. Upscaling these traditional processes requires overcoming challenges related to inconsistent outcomes, ensuring that consumers enjoy the same sensory experience both during and after consumption.

1.6 Research problem statement

While consumer attitudes and preferences for fermented foods and other functional foods have been extensively studied in other parts of Africa and around the world, there has been limited investigation into these aspects of fermented foods in the Zambian market (Adinsi et al., 2015; Akinsemolu, 2018). Sensory attributes such as flavor, appearance and consistency are important stimuli motivating consumer perception and their purchasing attitudes to a large extent. A previous study by Moonga et al. revealed that taste and consistency followed by product appearance were key attributes determining consumer preference (Moonga, 2019). However, this study only interviewed consumers without evaluating the actual mabisi products. Additionally, aroma plays an important and decisive role in consumer perception of fermented

products. Previous studies have also investigated the volatile organic compounds that make up the aroma profile of mabisi, revealing a diverse range of compounds (Moonga et al., 2020). However, the key aroma contributors otherwise known as odour-active compounds, which are perceivable by the human olfactory senses, have not been identified (Clark, 1998). These odour-active compounds can be leveraged to optimize the aromas of fermented products and support the efforts to upscale production. Shelf life is another important aspect of enhancing the value of food products. Given that there are no proper storage facilities in most rural households where mabisi production is widespread, mabisi usually is kept at ambient temperatures for several days either on the producers' or consumers' shelves. With several calls to upscale mabisi to meet consumers' demands and empower the local women producers and small-scale dairy farmers, it becomes imperative to understand the stability of certain quality parameters as a function of storage time. Similarly, the diverse microbial community of traditional mabisi offers much technological and commercial potential that could be harnessed to improve product quality and consistency. More specifically, these autochthonous mabisi microbes can have an essential role in product optimization through careful selection and design of starter cultures for mabisi production with predictable quality and safety.

However, to be able to do so, more investigations on relevant product characteristics are needed to provide the knowledge reflecting science-based evidence for product optimization and thereby enhance consumer confidence in traditional foods. Yet, as the market demand slowly increases, there remains a gap in understanding how these foods can be scaled up with minimal or no shifts from the unique traditional characteristics valued by consumers.

By assessing consumer sensory perceptions, identifying key volatile compounds imparting specific aromas, understanding product storability, and the potential of autochthonous strains from traditional mabisi as fermentation starters, we can better understand crucial product

attributes that can drive innovation around traditional fermentation. The knowledge of these aspects of the product characteristics will help preserve the unique volatile and microbial fingerprints of mabisi. This will further provide an understanding of process dynamics over time and identify the microbes responsible for targeted volatile compounds. Our findings will augment our understanding of traditional fermentation offering applications for ecology-driven process design necessary for upscaling and optimization of traditional fermented products.

1.7 Objectives of the study

Traditional mabisi plays an important role in preserving the social and cultural values of rural communities while supporting local economies and promoting sustainable agricultural practices. The work of this thesis seeks to deepen our understanding of this traditional dairy product, shedding light on its unique attributes that can be used as potential avenues for improving its production. Figure 4 illustrates the thesis's schematic overview and objectives. In short, this study aimed to:

1. Determine the sensory properties of mabisi and understand the attributes that influence consumer acceptance of four variants of traditional mabisi.

2. Describe the volatile organic compounds (VOCs) and identify odour-active aroma compounds of four variants of mabisi using Gas Chromatography-Olfactometry-Mass Spectrometry (GC-O-MS).

3. Assess the influence of storage temperature on the shelf life of two variants of mabisi and how product functionality is affected.

4. Isolate autochthonous microbial strains from traditional mabisi and determine their acidifying potential and ability to produce volatile organic compounds in UHT milk.

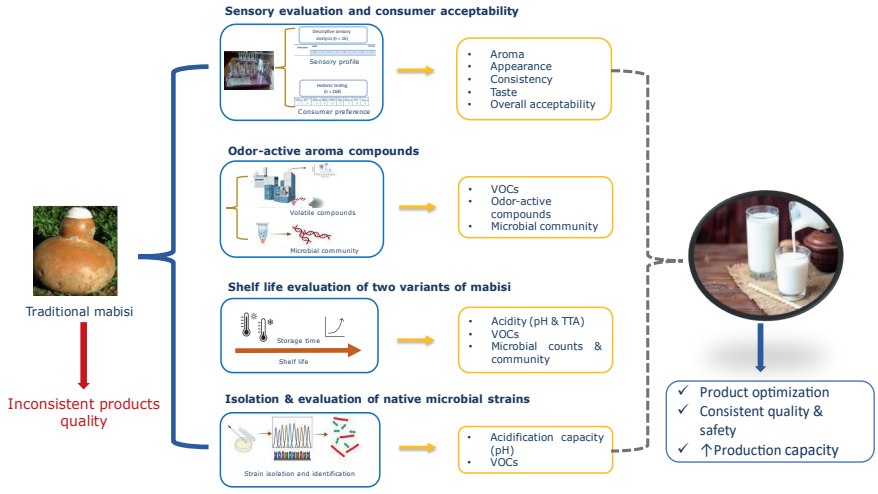

Fig. 4. Schematic presentation of the thesis chapters.

1.8 Outline of the thesis

In this thesis, we studied the characteristics of traditional fermented dairy products using mabisi from Zambia as an archetypical example. We begin with a general introduction to provide background information on traditional fermentation, focusing specifically on mabisi. The introduction also highlights the relevance of this study, outlines the problem statement, and details the study's objectives (**Chapter 1**). Further, we profile the sensory characteristics of mabisi using a semi-trained panel of judges to generate and evaluate the important descriptive sensory terms. The study focuses on four variants of mabisi, each distinguished by differences in production methods. Through sensory analysis and consumer acceptability evaluation in four districts of Zambia, we elucidate the nuances in aroma, taste, texture, and overall acceptability of these variants, discerning the perceptions of consumers and the drivers for their preferences (**Chapter 2**). Furthermore, the thesis investigates the odour-active components of mabisi, unravelling the interplay of volatile organic compounds that contribute to its unique aroma

characteristics. Using gas chromatography-olfactometry-mass spectrometry (GC-O-MS), we identify key aroma compounds and elucidate their sensory impact on the product (**Chapter 3**). In addition, this study explores the effects of storage temperature on the shelf life and functionality of two variants of mabisi, namely tonga and barotse. By subjecting the variants to varying storage conditions mimicking typical storage conditions available to the mabisi producers and consumers, we assess changes in physicochemical and microbial composition and the volatile compounds over time (**Chapter 4**). In the final research chapter **(Chapter 5)**, the thesis delves into the microbial dynamics of mabisi, with a focus on the isolation and identification of indigenous microbial strains. By harnessing these microbial resources, we demonstrate how to develop fermentation cultures with the potential to mirror the functional properties of the traditional variant, paving the way for future innovations in dairy product development. The performance of the isolated strains was evaluated both as monocultures and in mixed cultures, focusing on their acidifying potential and ability to produce volatile organic compounds in UHT milk. Finally, we integrate the findings of the research chapters in a general discussion and provide future perspectives to improve mabisi production with the ultimate goal of maximizing its potential for economic growth, and food and nutrition security (**Chapter 6**).

1.9 General description of the INREF project

This thesis was completed in the context of a Wageningen University Interdisciplinary Research and Education Fund (INREF) project, entitled '*Traditional fermented foods to promote food and nutrition security in Africa; entrepreneurship, value chains, product development and microbial ecology in Zambia, Zimbabwe and Benin*' (acronym: FermFood). The project targeted (a) upgrading food and nutrition security in Africa by (b) ameliorating the quality and use of traditional fermented foods through (c) strengthening the connected local

value chains and (d) fostering women's entrepreneurship. Since its start, the project has funded 8 PhD trajectories and 1 postdoc position.

The project had three main specific objectives linked to the fourth objective of interdisciplinary integration (Fig. 5). Three traditional fermented foods were selected as representative examples, namely the dairy-based *mabisi* in Zambia, the cereal-based *mahewu* in Zimbabwe and the cereal-based *akpan* in Benin. Together these products cover different aspects, e.g., rural versus urban, and the current levels of standardization and contribution to diets. This range maximizes the relevance of our research and helps to expand findings to other traditional fermented foods and African food systems in general. In essence, scientific output will serve as a blueprint of the current reality and solutions to allow findings to be widely applied in Africa and beyond.

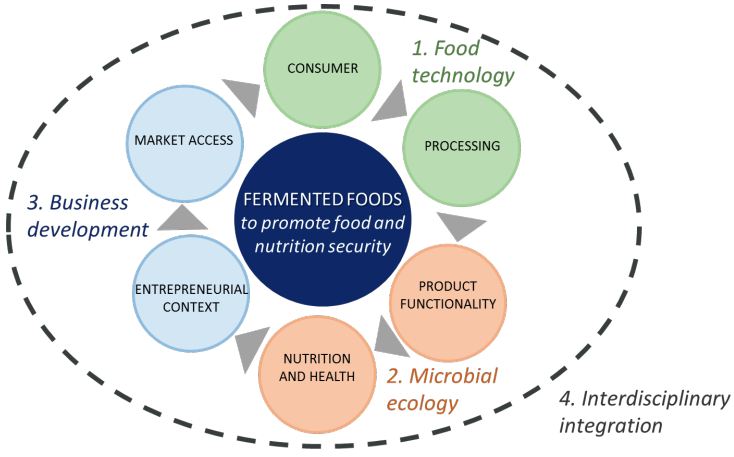

Fig. 5. Overview of the research activities and linkages in the FermFood project

This thesis was conducted under the "Upgrading of traditional food processing" objective using mabisi, a traditional dairy product from Zambia. Traditional mabisi is still at the household

production level but has exhibited potential for growth due to its perceived health benefits. Thus, in this thesis, the needs of consumers regarding sensory attributes that influence their acceptability of mabisi were explored. This also included identifying key odourants that the human olfactory senses can detect. The thesis further considered other characteristic properties of mabisi that can contribute to product optimization and scaleup of mabisi production to promote the development of traditional enterprises and benefit the producers and consumers. Since rural communities have challenges with cold storage facilities, the shelf life of the traditional product under ambient temperatures was examined. Furthermore, the potential of using autochthonous mabisi microbes as starter cultures to initiate the fermentation process and ensure predictable and consistent product outcomes was assessed. It is envisaged that these aspects of mabisi could support the efforts of upgrading traditional food processing and contribute to rural development.

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CHAPTER

2

Sensory characteristics and consumer acceptability of four variants of mabisi, a traditionally fermented Zambian dairy product.

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This chapter has been published as:
Sikombe, T. W., Moonga, H. B., Schoustra, S. E., Shindano, J., Stieger, M., Smid, E. J., & Linnemann, A. R. (2023). Sensory characteristics and consumer acceptability of four variants of mabisi, a traditionally fermented Zambian dairy product. LWT, 188, 115410. https://doi.org/10.1016/j.lwt.2023.115410

Abstract

Consumer acceptability of four variants of mabisi, a traditional fermented Zambian dairy product, was determined as a first step towards identifying directions for improving and stabilizing consumer-oriented product quality. The four mabisi variants differed in their production methods and associated sensory properties. The variants were tonga, backslopping, barotse and illa mabisi, characterized by undisturbed fermentation, using part of a previous batch, removing whey followed by the addition of fresh milk and vigorous shaking, respectively. Sixteen panelists generated and evaluated the intensity of ten descriptive sensory terms while 268 consumers rated their preferences for overall liking and five other hedonic attributes. Significant differences ($p<0.05$) were observed between the four variants for all sensory attributes except fermented odour. The products were distinguished by the textural attributes of thickness, smoothness, and creaminess. A multifactor analysis further demonstrated that textural attributes were the drivers of mabisi liking. When clustered according to overall liking, consumers were segmented into 3 clusters namely barotse-tonga preference (n=94), absolute barotse preference (n=84), and backslopping-illa-tonga preference (n=90). Overall, our findings show that barotse and tonga mabisi were more appealing to larger consumer segments, warranting further research into the optimization of product quality.

1.0 Introduction

Fermentation is an ancient method of preservation for highly perishable foods by the growth of beneficial microorganisms that eliminate or inhibit the growth of harmful microorganisms through the secretion of antimicrobial agents such as organic acids and bacteriocins (Fellows, 2000; Hui, 2012). Fermented milk products are among the most common fermented foods, dating back to ca. 6000 years BC (Kurmann, 1992). Fermented milk is produced by bacteria, mainly lactic acid bacteria, which break down lactose to lactic acid thereby reducing the pH of the milk and forming a gel-like body (Fox, 2011). Consequently, fermentation inhibits the growth of many pathogenic and spoilage bacteria, thus increasing the microbial safety of the product and extending its shelf life for several weeks (Fox, 2011). Fermentation creates attractive and appealing organoleptic properties that include improved texture and flavor of the product. Additionally, the nutritional value of foods is enhanced through mechanisms such as the biosynthesis of vitamins (Caplice & Fitzgerald, 1999).

A widely consumed fermented milk product in Zambia is mabisi. Mabisi is produced at the household level following the spontaneous fermentation of raw bovine milk. It is valued for its contribution to nutrition, the culture, and diversity of local foods and is produced at a small scale level, in quantities only sufficient for home consumption and to supply the local communities. Moonga and colleagues described seven production methods for traditional mabisi, each of which creates a distinct type of mabisi with unique sensory properties (Moonga et al., 2019). The production methods for mabisi vary depending on regional and producer preferences, but all involve an initial spontaneous fermentation of raw bovine milk at ambient temperatures for 24 - 48 h or until the milk sours and coagulates into a thick curd. When the fermentation is terminated at this stage, the mabisi produced is referred to as tonga type mabisi (Moonga et al., 2019). However, raw milk is sometimes inoculated with a small portion of a previously successful batch in what is known as the backslopping method (Holzapfel, 1997;

Ray, 2015), to produce a variant of mabisi referred to as backslopping type. Variations in the production methods range from the alternate removal of whey and the addition of milk to vigorous agitation to the point of butter formation that is later removed to produce barotse and illa types of mabisi, respectively. Other common types of traditionally produced mabisi include thick-tonga, creamy, and cooked mabisi (Moonga et al., 2019). Despite the different production methods, traditional mabisi sold in the markets is simply referred to as mabisi, without any method-associated distinctions.

Product properties such as consistency, pH, volatile profile, and microbial community composition largely depend on variations in processing (Moonga et al., 2019; Moonga et al., 2020). Batch-to-batch variations are inevitable in the final mabisi products, as the fermentation process is spontaneous with no control over the processing conditions. In other words, the quality of the final product is not entirely predictable because it depends on several uncontrolled factors such as the quality of the raw material, the fermentation temperature, and other environmental conditions. These factors dictate the type of microorganisms that participate in the fermentation and instigate metabolic reactions that, for example, produce the volatile and non-volatile compounds responsible for the flavor and taste (Cheng, 2010; Leroy & De Vuyst, 2004; Moonga et al., 2021; Ott, Hugi, Baumgartner, & Chaintreau, 2000; Smid & Kleerebezem, 2014) and thus the sensory properties and appeal of mabisi. The presence of volatile compounds such as organic acids, esters, ketones, alcohols, aldehydes, and sulphur compounds, and non-volatiles such as free fatty acids, peptides, and amino acids has been reported in fermented milk products, including mabisi (Dan et al., 2019; Dan et al., 2017; Moonga et al., 2020; Routray & Mishra, 2011; Smid & Kleerebezem, 2014).

In the last few years, the consumption of mabisi has expanded and spread to urban communities and areas not originally known to be mabisi-consuming communities. This is evidenced by the increase in both traditionally and commercially produced mabisi products on the open markets

and in supermarkets (Moonga et al., 2022). This expansion could at least partially be due to consumers' awareness of the relationship between food and health, and an increased preference for native and local Zambian foods. Regarding health benefits, this concerns improved digestion and the anti-inflammatory effects of fermented foods, which are important for well-being and the prevention of diseases (Szutowska, 2020). The increased consumer knowledge of food and health is attributed to better information availability through improvements in access to online sources of information, for instance, through smartphones. The growing demand for mabisi consumption is a motivation to increase its production. However, as consumers become more aware of their food environment, their expectations for product quality and safety also increase. These, in turn, call on producers to improve the production and quality of the traditionally fermented mabisi.

Improving the production and quality of traditionally fermented food products contributes to the sustainability of food systems that support the food and nutrition security of consumers and the livelihoods of processors (Materia, Linnemann, Smid, & Schoustra, 2021). Knowledge of consumer preferences is crucial for the success of any product development and improvement venture, and sensory properties are important regarding product quality. A study by Moonga and colleagues demonstrated that variations in the production methods affect the taste, consistency, and appearance of mabisi (Moonga et al., 2019; Moonga et al., 2022). However, the sensory characteristics of mabisi and their impact on product acceptance are underexplored. Therefore, establishing the sensory characteristics and understanding consumer perceptions of mabisi is necessary.

This study aimed to determine the sensory properties of mabisi and understand the attributes that influence consumer acceptance. A quantitative descriptive sensory analysis using a panel of semi-trained assessors and a consumer acceptance evaluation was conducted to describe the sensory characteristics and determine consumer preferences for four commonly consumed

types of mabisi (i.e., backslopping, barotse, illa, and tonga). Before this, a discrimination test was performed to ascertain sensory differences between the products. The results of this study form the basis for recommendations concerning optimizing the production and improving the quality of mabisi.

2.0 Materials and methods

2.1. Mabisi sample preparation

Four variants of mabisi, namely backslopping, barotse, illa, and tonga, were used in this study. The four mabisi variants were selected based on their popularity across the country (Moonga et al., 2019). Mabisi samples were prepared in the kitchen of the Food Science and Nutrition Department of the University of Zambia (UNZA), following the traditional methods of production described in a previous study (Moonga et al., 2019).

Each batch of the mabisi variants was produced from fresh raw cow milk collected from the UNZA field station. First, raw milk was sieved using a stainless steel sieve into plastic containers, covered with a lid, placed in a cool dry place, and left to ferment spontaneously. After 48 h of fermentation when a thick curd was formed, the process was terminated for tonga mabisi. Next, a small amount of the fermented product was used as an inoculum to start another fermentation cycle in the production of backslopping mabisi. This was repeated for two more cycles and the backslopping mabisi was ready. The production of illa mabisi was similar to that of backslopping, except illa production involved the vigorous shaking and agitation of the product after fermentation for about 20 min until butter granules were formed and settled on top of the fermentation container. The butter produced was scooped off and what remained in the fermentation container was the illa mabisi. For barotse mabisi, milk was left to ferment for 2 to 3 days until about 30 - 50% whey separation was evident. The whey was removed and more fresh raw milk was added. This was repeated for 3 more cycles before the product was ready as barotse mabisi.

2.2.Ethical approval

The use of human subjects in this study was approved by the Research Ethics Review Committee of the Tropical Diseases Research Centre, Ndola, Zambia (TRC/C4/24/2021). The communities where the study was conducted, were informed of the study through their civic leaders before the study commenced. Participants and consumers were briefed about the purpose of the study to allow them to make an informed decision. At the beginning of the study, each participant signed a consent form to participate in the study.

2.3. Proximate and physicochemical analysis

The proximate and physicochemical analyses were performed according to the AOAC official methods. pH was measured by a digital pH meter (Hanna HI 8424) and Total acidity was analyzed according to the AOAC official methods (AOAC, 2005). Acidity was calculated as a percentage of lactic acid after titrating 10 ml of mabisi against 0.1 N standard NaOH to a faint pink color that persisted for 30 sec. Consistency was determined with Adam's consistometer, which measures the diameter of the spread of a semi-liquid product after 30 sec (Barrett et al., 1998; Gould, 1992).

2.4. Discrimination test

Twenty-four participants (11 males, 13 females: age = 31.4±10.9 yrs.) were recruited to evaluate the samples of the four variants of mabisi. The participants were staff members and students of the University of Zambia who showed interest in participating in the study. All the recruited participants had prior experience with sensory evaluation on different products and were familiar with mabisi. A difference test using the triangle method was employed (Lawless & Heymann, 2010). Participants were given specific instructions regarding the triangle test before the commencement of the evaluation. Individual participants were presented with a set of three samples, two of which were similar and one was different. The samples were coded

with three-digit random numbers and presented in a random order to each participant with instructions on the appropriate order of assessment. The participants were asked to identify the odd sample out of the three. Along with the samples, each participant was provided with a score sheet and pencil to record their evaluation. Bottled water was available for participants to cleanse their palates after each sample evaluation.

A total of 6 triangle tests were presented to each of the participants with the following combinations in a randomized order: AAB, AAC, AAD, BBC, BBD, and CCD (with A: *backslopping*, B: *barotse*, C: *illa*, D: *tonga*). Between each of the two products compared, there were six possible serving orders, for instance, products A and B had the following possible combinations: AAB, ABA, BAA, BBA, BAB, ABB. Similar permutations were prepared for the other pairs of samples. The samples were balanced by having the triangles for the comparisons divided into two segments to give 2 sets of 24 tests, that is, when comparing A with B, half the participants received products from segment 1 as AAB samples with their respective permutations and likewise, the other half of the participants got products from segment 2 as ABB samples. A similar pattern was followed for the other product combinations. Each participant received a set of 6 triangles in one session presented as one set at a time. The evaluation took place over a session of 2 h with a break of 15 min mid-way.

2.5. Quantitative descriptive sensory analysis

The descriptive sensory evaluation was carried out in one of the lecture rooms at UNZA, at ambient temperatures (22 - 27 ºC) using normal lighting conditions. The room had sufficient space between the panelists to minimize interactions during sample evaluation. A modified quantitative descriptive analysis (mQDA) with a panel of semi-trained assessors was employed for the descriptive analysis. The panel comprised 16 participants (7 females and 9 males; age = 23.2 ± 1.6 yrs.), undergraduate students from the UNZA Food Science and Nutrition

department. Panelists were selected based on their familiarity with the mabisi product, willingness and availability to participate, and ability to discriminate between the samples. Their ability to discriminate between the samples was established through the triangle test described in section 2.4. Training of assessors and product evaluation were conducted in 8 sessions of 60 – 90 min each over 4 consecutive weeks with bi-weekly sessions. During the first session, the panelists received an orientation to familiarize them with the products, QDA methodology, the use of the scale, and the mouth cleansing procedure. The training also focused on generating descriptive and anchor terms for evaluating the mabisi products. During the next 4 sessions, panelists developed and defined the descriptive terms that were used in the evaluation of the mabisi products. The panelists and the sensory research leader discussed and assigned reference materials for each descriptive term generated.

Table 1. Descriptive terms with their respective definitions and reference materials as generated by the panellists

Descriptors	Definitions	Reference material
Appearance		
White color	A uniform appearance of white throughout the sample	Pure white cotton balls
Creamy	A consistent, thick, and viscous appearance	Double cream natural yoghurt
Taste		
Sour	A tart taste sensation characteristic of lemon	0.03% citric acid solution
Sweet	A taste sensation that is associated with sugar	2% sugar solution
Bitter	A pungent, unpleasant taste sensation typical of quinine	0.01% quinine sulfate solution
Aftertaste	A light, unusual sensation that lingers on the tongue after a sample is swallowed or expectorated	An unripe banana
Aroma		
Fermented	An aroma characteristic of activated yeast fermented products	0.8% yeast in 4% sugar solution left to stand overnight
Consistency (Texture)		
Creamy	A consistent, thick, and viscous feeling in the mouth	Double cream natural yoghurt
Thick	Related to the consistency of a sample that is difficult to flow	Double cream natural yoghurt
Smooth	An even consistency that is free of lumps throughout the sample	Drinking yoghurt

During the descriptive terms generation session, panelists were presented with the four mabisi variants and asked to list the sensory notes perceived for the products' appearance, aroma, taste, and both oral and visual texture (consistency). Seventeen sensory attributes were initially developed and from these, 10 descriptive terms that the panelists perceived to best define the products, were selected through consensus (Table 1). For the proper sensory evaluation, 30 mL mabisi at room temperature was presented in clear plastic cups labeled with 3-digit random numbers and covered with aluminum foil to avoid the volatilization of aroma compounds. The evaluation was performed by using a paper scoresheet containing the 10 descriptive terms on a 9-point structured intensity scale with each attribute anchored on the terms weak and strong at the lower and higher end, respectively. The panelists evaluated each of the products and rated the intensity of the sensory descriptive terms using the provided scale. Each sample was evaluated in triplicate for all 10 attributes.

2.6. Consumer preference survey

A consumer survey was carried out to evaluate consumer acceptability for the four mabisi variants. The survey was conducted in four districts in Zambia where mabisi production and consumption have been reported. A total of 268 consumers of mabisi of at least 18 years participated in the survey from the districts that included Lusaka in Lusaka province (n = 106), Choma and Namwala in Southern province (n = 81), and Mongu in Western province (n = 81) (Fig. 1). Two to three central locations were chosen in each district to conduct the consumer evaluations of the mabisi variants.

Samples were prepared and presented as described in section 2.5. Consumers received the four mabisi samples coded with 3-digit random numbers in a randomized order. Bottled water was provided as a cleanser between samples. A 9-point hedonic scale anchored on the terms “dislike extremely” scored as 1 to “like extremely” scored as 9 was used by the consumers to evaluate

each of the four mabisi variants for overall liking, appearance liking, aroma liking, taste liking, and oral and visual consistency liking (Balthazar et al., 2018; Meilgaard, Carr, & Civille 2007). Before the hedonic rating, consumers were asked to provide information on their socioeconomic status and their preferred category of mabisi between the traditionally and commercially produced types. The consumers were segmented into age groups, namely those in their 20s, 30s, 40s, 50s, and above 60 years. An electronic data entry application tool, CSentry 7.7.1 APK for Android was used to record consumer responses.

Fig. 1. Map of Zambia showing the four districts, denoted by the blue dots, where mabisi consumer survey was conducted.

(Map adapted from https://commons.wikimedia.org/wiki/File:Zambia_provinces_named.png)

2.7. Statistical analysis

The discrimination test data were analyzed using the statistical tables for the critical number of correct responses for estimating significance in triangle tests as given by Roessler and colleagues (1978). ANOVA was performed on the descriptive sensory analysis data to test for significant differences in the sensory attributes of the four products. In the case of significant differences among the attribute intensities, Tukey's HSD test ($p<0.05$) was conducted as a post hoc test to compare the differences between products. The descriptive analysis data was further analyzed with principal component analysis (PCA) to visually evaluate the differences and similarities between products and sensory attributes.

ANOVA followed by the Tukey HSD test was applied to the overall product acceptability ratings to test for significant differences among the consumer responses. Spearman's rank correlation coefficient was used to describe the correlation between overall product acceptability and the other hedonic attributes. Multi-factor analysis (MFA) was performed on the mean intensity values of the descriptive sensory analysis to assess the relationships between the sensory attributes and consumer acceptability. The descriptive sensory data was used as the active variables and the consumer acceptability data was used as supplementary variables. To divide the consumers into segments in which they shared a common preference pattern for the mabisi products, hierarchical cluster analysis was performed on Euclidian distances with Ward's minimum variance criterion using the overall product acceptability data. For the demographic data of consumers, the frequencies of the responses were calculated and analyzed by using the Chi-squared test of independence analysis. ANOVA was applied to the proximate and physicochemical data followed by the Tukey HSD test. Statistical analysis was performed by using XLSTAT statistical tool in Excel (ver. 2016) and R software (ver. 4.2.1). The SensoMineR package in R was used for the analysis of sensory and consumer hedonic data. Alpha ($\alpha = 0.05$) was used as the level of significance for all the statistical analyses.

3.0 Results

3.1 Proximate and physicochemical properties of the mabisi variants

The proximate and physicochemical composition of the four mabisi variants was determined (Fig. 2). The barotse variant had the highest dry matter, protein, and fat content, and a lower diameter of spread, indicating a relatively thicker and viscous consistency. Significant differences ($p<0.05$) were exhibited in the dry matter content, carbohydrates, and the diameter of the spread between barotse and the other three mabisi variants. The fat content of barotse type mabisi also differed significantly from the illa type mabisi. Illa had the highest diameter of spread and was thus less viscous than the other three variants, while tonga and backslopping types were similar regarding their viscosity and dry matter. The pH, titratable acidity, and ash content were similar for all four mabisi variants. Although the acidity for the barotse, illa, and backslopping types did not differ significantly, the tonga type had a significantly lower acidity than the barotse type.

3.3. Discrimination test

Discrimination tests using the triangle method were performed on the four variants of mabisi to assess whether the products differed organoleptically (Fig. A1). Each of the 6 triangle tests revealed significant differences ($p < 0.01$) between the mabisi samples. This demonstrates that the four mabisi variants, i.e., backslopping, barotse, illa, and tonga, differ significantly from each other in their sensory perception.

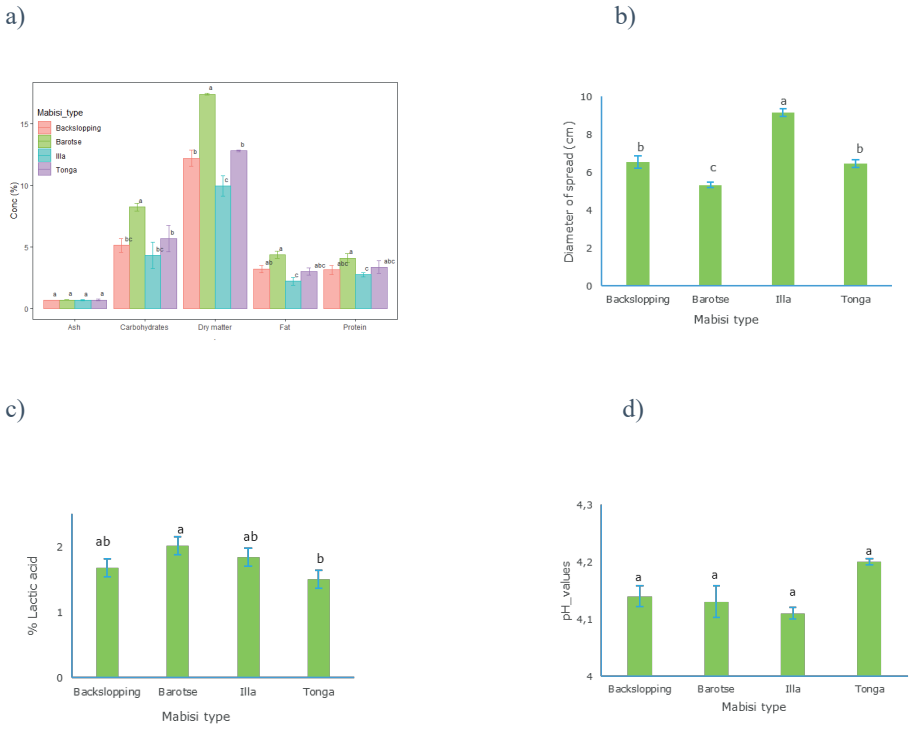

Fig. 2. Proximate composition (a) and physicochemical characteristics of product consistency (b), acidity (c), and pH (d) of the 4 variants of traditional mabisi.

3.4. Sensory characteristics of the four variants of mabisi products

The output of the ANOVA tests performed on the quantitative descriptive analysis data revealed significant differences ($p<0.05$) among the products for all the sensory descriptors except for one attribute, namely fermented odour. Barotse was rated highly for its appearance attributes, for instance, creamy appearance was significantly higher in barotse than in the other 3 mabisi variants although the white color in barotse did not differ significantly from that of the tonga mabisi. While no difference was observed between the tonga and backslopping mabisi concerning appearance attributes, illa was rated the lowest of the four variants.

Table 2. Mean intensity scores with standard deviations (n=16) of the ten sensory descriptive terms for the mabisi variants.

Attributes	Backslopping	Barotse	Illa	Tonga	F values	p-values
Appearance						
White color	$4.6 \pm 1.8^{a*}$	6.0 ± 2.0^{b}	4.1 ± 2.2^{a}	4.9 ± 2.0^{ab}	4.443	0.000
Creamy appearance	4.6 ± 1.8^{a}	6.6 ± 2.0^{b}	3.0 ± 2.0^{c}	5.1 ± 2.0^{a}	7.943	0.000
Aroma						
Fermented odor	3.8 ± 2.0	4.6 ± 2.2	3.9 ± 1.9	3.9 ± 2.0	1.286	0.099
Taste						
Sour taste	5.0 ± 2.1^{ac}	5.7 ± 1.9^{ab}	6.2 ± 2.1^{b}	4.1 ± 2.1^{c}	4.804	0.000
Sweet taste	4.2 ± 2.0^{a}	3.1 ± 1.7^{b}	4.2 ± 2.5^{a}	3.5 ± 1.9^{ab}	2.209	0.014
Bitter taste	3.3 ± 2.3^{a}	4.2 ± 2.4^{ab}	4.7 ± 2.5^{b}	3.3 ± 2.0^{a}	3.124	0.001
Aftertaste	4.2 ± 2.3^{a}	4.5 ± 2.4^{ab}	5.5 ± 2.3^{b}	4.1 ± 2.3^{a}	2.688	0.004
Texture						
Creamy consistency	3.5 ± 2.0^{a}	6.0 ± 2.1^{b}	2.9 ± 2.0^{a}	5.0 ± 2.0^{b}	7.823	0.000
Thick consistency	3.2 ± 1.6^{a}	7.1 ± 1.7^{b}	1.8 ± 1.0^{c}	5.2 ± 1.7^{d}	14.246	0.000
Smooth consistency	4.6 ± 1.7^{a}	2.1 ± 1.5^{b}	6.1 ± 2.0^{c}	3.9 ± 1.9^{a}	10.888	0.000

**Different superscript letters within a row indicate significant differences.*

Thickness was significantly different among the four mabisi variants; barotse was perceived to have the thickest consistency followed by tonga, and finally backslopping and illa being the least thick variants. Regarding the creamy consistency, barotse and tonga were perceived to be similar in creaminess and significantly more so than backslopping and illa. Illa was rated significantly higher concerning smoothness while backslopping and tonga had similar ratings and barotse was the least smooth product. For the taste attributes, illa was perceived to be more sour than the tonga and backslopping mabisi but did not significantly differ from the barotse. The intensity of the sweet and bitter tastes was comparatively weaker than the other attributes for all the mabisi variants, with barotse having a significantly less intense sweet note. Similarly, bitterness was perceived more in the illa mabisi, followed by barotse, and least in the tonga and backslopping variants.

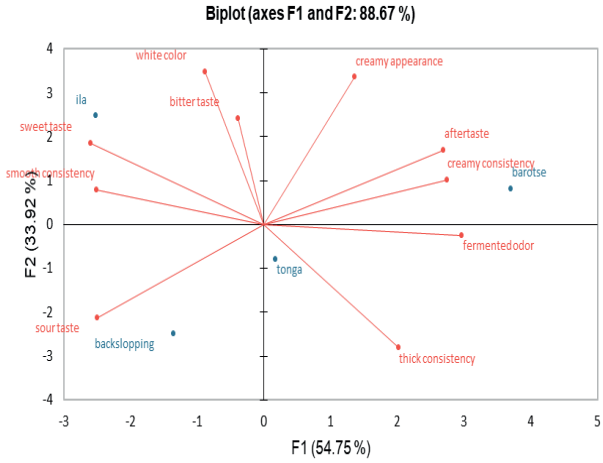

Fig. 3. Bi-plot from principal component analysis (PCA) of the descriptive sensory data showing the correlation between sensory attributes and the four variants of mabisi. The x- and y- axes represent the first two components of data explained by a total variance of 88.6 %.

A total variance of 88.6% was explained by the first two principal components with 54.7% and 33.9% for PC1 and PC2, respectively (Fig. 3). The four products were clearly separated from each other as demonstrated by the PCA. Barotse, which was positively projected on PC1, was very distinctive from backslopping and illa and to some extent from the tonga mabisi. This distinction is attributed to the contrast observed in their creamy and thick consistency attributes, as these attributes were more closely associated with barotse than backslopping and illa mabisi. On the contrary, illa loaded on the negative direction of PC1 and was associated with a smooth consistency and the taste attributes, bitter, sweet, and sour taste. While the backslopping mabisi correlated more with the sour taste on the negative direction of PC2, it also shared some similarities with the illa type in the aspect of the sweet taste. Tonga, on the other hand, was projected on the negative direction of PC2 but positioned very close to the center relative to the other variants that were clearly further apart and away from the center.

PC1 shows a high correlation between the aftertaste, fermented odour, and creamy consistency and appearance. Similarly, the thick consistency correlated highly with the creamy consistency and the fermented odour. On the other hand, sweet taste and smooth consistency were highly correlated on the negative dimension of PC1, while the white color and bitter taste had a strong correlation and were projected on the positive dimension of PC2.

3.5 Consumer acceptability of mabisi

Table 4 presents a summary of the mean consumer acceptability scores on a 9-point hedonic scale. Overall, barotse and tonga mabisi received higher liking scores than backslopping and illa types. Barotse and tonga mabisi had mean scores ranging between "like slightly" (score = 6) and "like moderately" (score = 7) whereas backslopping and illa had mean scores that ranged between "neither like nor dislike" (score = 5) and "like slightly" (score = 6). Barotse and tonga types were rated the highest in terms of overall liking and oral consistency liking. Nevertheless, all four products received mean scores above 5 but below 7 on a 9-point hedonic scale, which in general terms is regarded as acceptance for the products. Barotse was the most preferred variant on all the hedonic attributes except for the aroma attribute where tonga received the highest score although the difference was not significant. A similar pattern of liking for the aroma was observed in all four variants, but illa had a significantly lower score than tonga mabisi ($p = 0.016$). Overall, the liking scores for the illa and backslopping mabisi were trailing behind the other two variants on all the evaluated attributes. Backslopping was the least preferred variant on overall product acceptability and appearance, while illa was rated the least on the other four attributes including aroma, taste, and the oral and visual consistency. However, overall consumer liking for illa and backslopping mabisi did not differ significantly from one another.

Table 3. Mean consumer preference scores on a 9-point hedonic scale with standard deviations (n = 268) for overall acceptability and five hedonic attributes for the mabisi variants.

Attributes	Backslopping	Barotse	Illa	Tonga	F- value	p-value
Overall liking	$5.6 \pm 2.4^{c*}$	6.4 ± 2.9^{a}	5.7 ± 2.4^{bc}	6.2 ± 2.5^{ab}	6.110	0.000
Appearance liking	5.8 ± 2.0^{b}	6.9 ± 2.7^{a}	5.8 ± 2.5^{b}	6.3 ± 2.4^{b}	13.588	0.000
Aroma liking	6.0 ± 2.2^{ab}	6.1 ± 3.0^{ab}	5.8 ± 2.2^{b}	6.5 ± 2.5^{a}	3.451	0.016
Oral consistency liking	5.7 ± 2.1^{b}	6.4 ± 3.0^{a}	5.6 ± 2.3^{b}	6.3 ± 2.5^{a}	7.791	0.000
Taste liking	5.9 ± 2.2^{ab}	6.4 ± 3.0^{a}	5.7 ± 2.4^{b}	6.3 ± 2.4^{a}	4.814	0.002
Visual consistency liking	5.7 ± 2.1^{bc}	7.0 ± 2.7^{a}	5.5 ± 2.4^{c}	6.2 ± 2.4^{b}	21.661	0.000

**Different superscript letters within a row indicate significant differences.*

3.5.1 Linking consumer acceptability to the sensory attributes

The results of the multiple factor analysis (MFA) applied to the descriptive sensory data and the consumer hedonic ratings are presented in Fig. 4. Overall liking was highly loaded on the positive direction of Dim1 and correlated more strongly with white color, creamy appearance, and thick and creamy consistency. On the contrary, the overall liking exhibited a negative correlation with smooth consistency and sweet taste, and to some extent the other taste attributes. This suggests that the visual and textural attributes of creamy appearance, and thick and creamy consistency were the drivers for the overall liking of mabisi by consumers, whereas smooth consistency and sweet taste were drivers for dislike. Consistency, as described by the panelists in section 2.5, was characterized by a highly viscous and lumpy gel for barotse mabisi and a smooth and light gel for illa, while the tonga and backslopping mabisi were described by an intermediate textural consistency. Other than the overall acceptability and oral consistency, tonga and backslopping mabisi had similar liking patterns while backslopping was more similar to illa mabisi regarding the overall acceptability.

a) b)

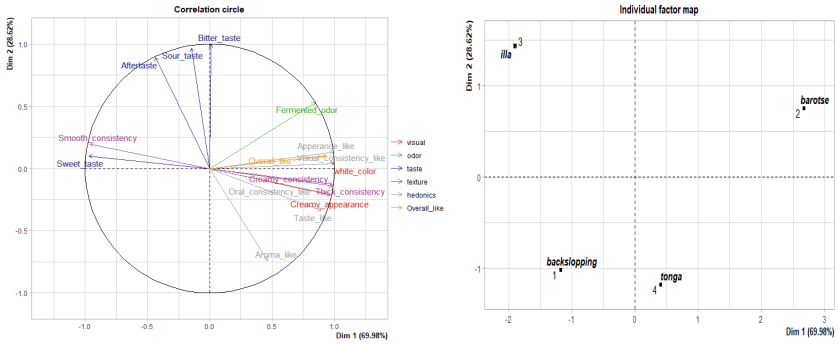

Fig. 4. Multiple factor analysis (MFA) plots showing the relationship between descriptive analysis (bold labels) and consumer acceptance test (orange and grey labels) for the four variants of mabisi (a) and the projection of the individual products on the factor map (b). The x- and y- axes represent the first two components of data explained by a total variance of 98.6 %.

3.5.2 Relationship between overall consumer acceptability and hedonic attributes

Spearman's rank correlation was performed on the consumer ratings for overall liking and the hedonic attributes of appearance, aroma, taste and consistency to identify the hedonic attributes that correlated most with the overall liking. For the barotse type, all the hedonic attributes correlated strongly with the overall liking ($r > 0.84$) and ($p < 0.001$). For the other three variants, a positive correlation was also observed between the overall liking and the hedonic attributes albeit not as strong as for barotse mabisi (Table A1). However, the appearance liking in the tonga mabisi had the weakest correlation and did not show a significant correlation with the overall liking ($r = 0.11$) and ($p = 0.068$).

3.5.3. Consumer segmentation

Cluster analysis was conducted on the overall product acceptability scores to classify and evaluate consumers based on their preference for the four mabisi variants. Three clusters of consumers were identified. An overview of the clusters was projected on principal components

comprising Dim 1 and 2 (Fig. 5). The first two principal components accounted for the largest portion of variability with a total of 64.9% (33.3% and 21.7% for the first and second dimensions, respectively). The first and largest cluster (n = 94) of consumers had a preference for tonga and barotse mabisi. The second cluster (n = 84) consisted of consumers with a sole preference for barotse, while cluster 3 (n = 90) comprised consumers that preferred tonga, backslopping, and illa mabisi (Table 4).

Table 4. Mean values of attribute scores for variants of mabisi by consumer clusters of preference.

Attributes	Cluster	Product			
		Backslopping	Barotse	Illa	Tonga
Overall acceptance***	1	$4.65^{a¥}$	7.83^{b}	4.96^{a}	7.20^{b}
	2	6.04^{a}	7.74^{b}	6.25^{a}	4.53^{c}
	3	6.10^{a}	3.84^{b}	6.08^{a}	6.64^{a}
Appearance***	1	5.18^{a}	8.04^{b}	4.96^{a}	7.06^{c}
	2	5.71^{ac}	7.93^{b}	6.26^{a}	4.87^{c}
	3	6.36^{a}	4.88^{b}	6.16^{a}	6.88^{a}
Aroma***	1	5.66^{a}	7.27^{b}	5.26^{a}	6.76^{b}
	2	5.74^{a}	6.92^{b}	6.09^{ab}	5.26^{a}
	3	6.71^{ac}	4.27^{b}	6.10^{c}	7.4^{a}
Taste***	1	5.60^{a}	7.43^{b}	5.07^{a}	6.72^{b}
	2	5.66^{a}	7.38^{b}	6.07^{a}	5.29^{a}
	3	6.34^{a}	4.36^{b}	6.00^{a}	6.92^{a}
Oral consistency***	1	5.35^{a}	7.64^{b}	4.90^{a}	6.79^{c}
	2	5.45^{a}	7.09^{b}	5.94^{a}	5.14^{a}
	3	6.31^{ac}	4.67^{b}	6.00^{c}	6.99^{a}
Visual consistency*	1	4.94^{a}	7.91^{b}	5.02^{a}	6.56^{c}
	2	5.85^{a}	7.64^{b}	5.52^{a}	5.36^{a}
	3	6.22^{ab}	5.52^{a}	5.86^{ab}	6.56^{b}

**Different superscript letters within a row indicate significant differences.*
*Significant difference codes: *p-value ≤ 0.05 **p-value ≤ 0.01 ***p-value ≤ 0.001*

Cluster 1 consumers had a high preference for barotse and tonga, and a lower preference for backslopping and illa mabisi. Cluster 2 had an exclusive preference for barotse, which was rated much higher than backslopping and illa mabisi. Tonga mabisi was the least preferred variant by cluster 2 consumers. The preference pattern for cluster 3 consumers comprised illa, backslopping, and tonga mabisi. These findings also indicate that backslopping and illa mabisi

were highly related regarding the overall product acceptance by consumers as demonstrated by their closeness in the PCA projection (Fig 5). Barotse was, however, the least preferred variant by cluster 3 consumers.

a) b)

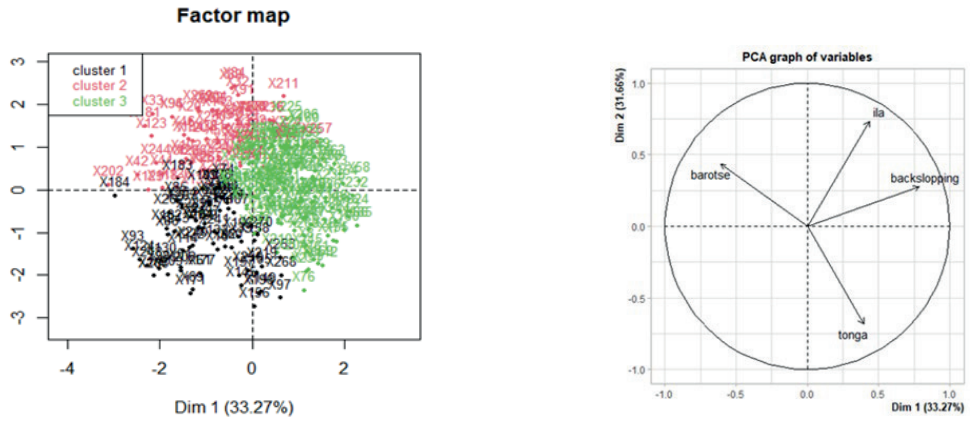

Fig. 5. Principal component (PC) scores for the overall product acceptability segmented into clusters of consumers exhibiting similar preferences for mabisi variants (a) and the projection of product loading on the PCA (b). The x- and y-axes represent the first two components of data explained by a total variance of 64.9 %.

Regarding the specific hedonic attributes for the mabisi variants, cluster 1 consumers exhibited a high preference for all hedonic attributes associated with the barotse and to a great extent tonga mabisi with a lower preference for the attributes related to backslopping and illa mabisi. Cluster 2 consumers showed a high preference for barotse mabisi and its associated hedonic attributes. Tonga mabisi and its hedonic attributes were least preferred by this cluster. In addition, cluster 2 consumers appeared to prefer the aroma of illa mabisi as much as the barotse aroma. This cluster also liked the appearance and taste of illa mabisi more than that of backslopping and tonga mabisi. In contrast, cluster 3 consumers liked all the hedonic attributes of backslopping and tonga mabisi the most and had the least preference for the hedonic

attributes associated with barotse mabisi. Between backslopping and illa, cluster 3 consumers liked the hedonic attributes associated with illa mabisi the least.

3.5.4. Characterization of the demographic profile of consumer clusters

A summary of the demographic profile of the consumers in the three preference clusters is given in Table 5. The clusters differed significantly in the proportion of consumers from the three different regions in the study as well as the marital status of the consumers (Chi-squared test, $P < 0.05$). For instance, cluster 1, with a preference for barotse and tonga mabisi, had a higher proportion of consumers from Lusaka than the other two regions. There were no significant differences in the proportions of consumers regarding their age, gender, educational background, employment status, family income, category of mabisi consumed, and frequency of consumption. Notably, there were more male consumers than females across all three clusters, and clusters 2 and 3 especially recorded a high male-to-female ratio (Table 5). Consumers in all three clusters were dominated by the 18 - 48 age group and most of them had some form of employment.

The majority of the consumers had at least a secondary level of education. The proportion of consumers with a preference for both commercial and traditional mabisi was greater than that of consumers whose preference was for either commercial or traditional mabisi. The different regions did not affect this observation, as this was the general pattern in all three regions. In addition, consumers who only preferred commercial mabisi comprised the smallest group in all the clusters. Finally, most respondents appeared to be regular consumers of mabisi with a consumption frequency of three or more times a week.

Table 5. Demographic characteristics of consumers based on three clusters of preference. The table shows the influence of consumer demographic profile on the preference attitude towards the mabisi variants.

Consumer profile	C1 (%)	C2 (%)	C3 (%)	χ^2 value[a]
N	33.7	32.2	34.1	
Gender				0.45
Female	49.4	44.7	45.6	
Male	50.6	55.3	54.4	
Age group (years)				14.94
18-29	30.3	31.8	45.6	
30-39	24.7	22.4	28.9	
40-49	22.5	20.0	17.8	
50-60	13.5	16.5	6.7	
>60	9.0	9.4	1.1	
Region (province)				13.1*
Lusaka	47.2	30,6	38.9	
Southern	16.9	37.6	36.7	
Western	36.0	31.8	24.4	
Marital status				14.5*
Divorced	3.4	1.2	3.3	
Married	59.6	65.9	47.8	
Single	40.4	34.1	52.2	
Widowed	2.2	8.2	2.2	
Education level				1.58
Primary	22.5	20.0	16.7	
Secondary	40.4	43.5	40.0	
Tertiary	37.1	36.5	43.3	
Employment status				4.67
Student	6.7	7.1	11.1	
Unemployed	36.0	28.2	32.2	
Employed	55.1	63.5	56.7	
Retired	2.2	1.2	0.0	
Family income				6.88
Less than K2, 000	51.7	51.8	54.4	
Between K2, 000 & K4,999	30.3	28.2	31.1	
Between K5,000 & K9,999	13.5	16.5	11.1	
Between K10,000 & K14,999	1.1	0.0	2.2	
Between K15,000 & K19,999	3.4	2.4	0.0	
Above K20, 000	0.0	1.2	1.1	
Category of mabisi consumed				2.73
Commercial & traditional	56.2	57.6	68.9	
Commercial only	19.1	15.3	8.9	
Traditional only	24.7	27.1	22.2	
Frequency of consumption				4.52
At least once a week	27.0	28.2	27.8	
Three or more times a week	27.0	35.3	32.2	
Once every two – three weeks	6.7	8.2	5.6	
At least once a month	20.2	17.6	15.6	
Occasionally, once in three months	19.1	10.6	18.9	

[a] *χ^2 Test to test the significance of difference between clusters*
** p_value ≤ 0.05*

4.0 Discussion

The panel of assessors in the discrimination test of our study perceived differences among the four mabisi variants, thus confirming that the products differed regarding their sensory properties. Different characteristics of mabisi influenced the level of consumer perception and liking of the products. The differences in the intensity of creaminess, thickness, and smoothness between the four variants were the largest, and differences in taste and appearance, although significant, were small in comparison to the textural attributes. Thus, the discrimination between the mabisi variants was driven by the textural differences. The most pronounced textural differences were observed between barotse and illa mabisi. The taste was another distinguishing characteristic with relatively more pronounced sour and bitter notes in the illa and barotse mabisi than in the tonga and backslopping mabisi. Interestingly, the fermented odour attribute did not significantly contribute to distinguishing the products despite a comparatively high intensity in barotse mabisi.

Our findings are partially in agreement with previous studies on mabisi and similar fermented dairy products. An earlier study by Moonga and colleagues reported that consistency and appearance were among the highest-ranked attributes by consumers in determining the best quality for mabisi. That study (Moonga et al., 2022) further indicated that taste was a highly ranked attribute, which was not the case for our study. This contradiction could be linked to the fact that the previous study only interviewed the consumers of mabisi for their perception and did not use actual mabisi samples in a sensory evaluation approach. Another consumer acceptability study of a similar traditional fermented milk from South Africa, amasi, had contrary findings where product color was highly ranked and a more preferred sensory attribute in comparison to sweetness, sourness, and texture (Moyane, 2013). Furthermore, other researchers working with yogurt have also reported that texture attributes such as creaminess, body, and consistency play an important and decisive role in the quality of the final product

(Ares, Giménez, & Gámbaro, 2008). Soukoulis and colleagues also corroborate the large influence that texture has on the acceptability of similar semi-solid dairy products although these were produced with the use of starter cultures. This underpins the significance of texture as a quality attribute for consumer acceptance and shows the importance of paying particular attention to this attribute in product optimization (Soukoulis, Panagiotidis, Koureli, & Tzia, 2007).

The differences among the mabisi variants are attributed to the use of different processing methods. For instance, the production of barotse mabisi involves the removal of the whey that forms during the fermentation process, leaving a highly viscous and lumpy gel. In contrast, the vigorous shaking and removal of the buttery layer that forms on top of the illa mabisi result in a smooth final product with a lighter consistency. The bitter taste observed in the illa mabisi could be due to the presence of bitter peptides or amino acids such as oligopeptides, tryptophan, and tyrosine (Bumberger & Belitz, 1993; Richter et al., 2022; Smid & Kleerebezem, 2014), as well as fatty acids, such as butanoic, hexanoic, octanoic, and decanoic acids. These compounds have been reported in mabisi products and other traditional fermented dairy products such as omashikwa from Namibia, and are known to be a result of the enzymatic breakdown of proteins and fats (Misihairabgwi & Cheikhyoussef, 2017; Moonga et al., 2019).

The technique of backslopping has been suggested as the best method to accelerate traditional fermentation processes and to improve the quality of spontaneously fermented products (Holzapfel, 1997; Holzapfel, Geisen, & Schillinger, 1995; Teniola, Holzapfel, & Odunfa, 2005). Backslopping affects the microbial dynamics, resulting in a more stable community of microorganisms after a few cycles due to the dominance of the best-adapted strains (Schoustra Kasase, Toarta, Kassen, & Poulain, 2013). In principle, the resulting product is expected to be more consistent and of better quality than the spontaneously fermented product. Interestingly, our study showed that the spontaneously fermented tonga mabisi was generally more appealing

to consumers than the backslopping variant. The preference for tonga over backslopping mabisi was observed for all the hedonic attributes evaluated. This finding could be attributed to the lower pH and relatively lower viscosity of the backslopping mabisi compared to the tonga variant. Backslopping mabisi has a higher viable bacterial cell count at the onset of fermentation compared to the tonga mabisi because an active culture is added from a previous batch of fermented product. Tonga mabisi fermentation, on the other hand, largely depends on the bacterial community present in the raw milk, which is relatively low in number (Groenenboom, Shindano, Cheepa, Smid, & Schoustra, 2020). The difference in the bacterial density at the onset of fermentation when comparing the two processing methods causes differences in the initial acidification rates. This potentially affects the water retention and gel formation kinetics and thus the textural properties of the two products (Priyashantha, Buldo, Berg, Gilleladen, & Ipsen, 2021). The differences in the consistency of these two variants of mabisi are in agreement with the results of Moonga et al. (Moonga et al., 2022). These findings stress the need for further investigations into the backslopping method of mabisi fermentation for an optimized product that is more acceptable to consumers as this is the basis for upscaling using starter cultures.

Consumers may perceive a product's sensory attributes differently (Péneau, Hoehn, Roth, Escher, & Nuessli, 2006). In our study, three clusters of consumers with distinct preference patterns for mabisi were found, with, by chance, comparable numbers of consumers in each cluster. Overall, barotse mabisi seemed to be the most preferred variant of mabisi as shown by the sole preference for this type by the consumers from cluster 2 in addition to a shared liking by consumers from cluster 1. Cluster 3 consumers liked backslopping, illa, and tonga mabisi more than barotse and its associated hedonic attributes. Interestingly though, the other three variants have unrelated sensory characteristics, and thus it is not exactly clear what attributes drive product liking in this consumer cluster. For instance, tonga mabisi is characterized by a

thick and somewhat creamy oral and visual consistency with a mellow sour and less sweet taste, whereas illa mabisi has a sharper sour taste and a much lighter consistency. Attributes of backslopping mabisi are between those of the illa and the tonga variants. Without a doubt, this implies that products with comparable liking scores may not necessarily have similar sensory characteristics (Kemp, 2009; Lawless & Heymann, 2010).

Consumer geographic location and demographics have also been demonstrated to influence the way consumers perceive the sensory properties of foods. Our findings indicate that only location, in this case the region where the interviews were conducted, and marital status, had a significant correlation with consumer preference for a particular mabisi variant. The significance of the region could be due to the different types of mabisi that consumers know and are familiar with (Moonga et al., 2019; Yang & Lee, 2019). This information could be useful in producing tailor-made mabisi products for specific regions of the country (Moonga et al., 2019).

Interestingly, the majority of consumers did not have a particular preference for either commercial or traditional mabisi but those who specifically preferred one category over the other, had reasons for their choice. Given reasons included convenience, availability, affordability, food safety, and the different flavor and aroma notes, describing traditional mabisi as natural and relatively rich and intense in flavor. Food safety is a serious concern for a majority of consumers, but a recent study suggests that the current traditional mabisi processing methods using raw milk lead to a microbiologically safe product, although some potential pathogens approach critical limits (Schoustra et al., 2022).

5.0 Conclusion

This study demonstrates how consumer preference for variants of traditional mabisi is significantly influenced by the product's sensory characteristics. Overall, consistency and

appearance seemed to have the greatest influence on the product and are therefore drivers of mabisi liking by consumers. Even though different groups of consumers have their specific preferred variants of mabisi, largely dependent on the region of the country, the findings generally indicate that barotse and tonga mabisi had more appealing attributes and hence were more preferred by a larger proportion of consumers. Thus, it would be imperative to focus on the attributes presented by these two mabisi variants for further product optimization and production upscaling endeavors.

Acknowledgements

We would like to express our gratitude to the individuals and institutions that supported this work. We thank the dairy cooperatives in Choma, officers from the Ministry of Livestock and Fisheries in Choma, Namwala, and Mongu districts of Zambia, officers from Chelstone Clinic in Lusaka, the interns from the National Institute for Scientific and Industrial Research and University of Zambia members of staff and students that participated in this study. This research was financially supported by the Integrated Research Fund (INREF) at Wageningen University and Research.

Supplementary material

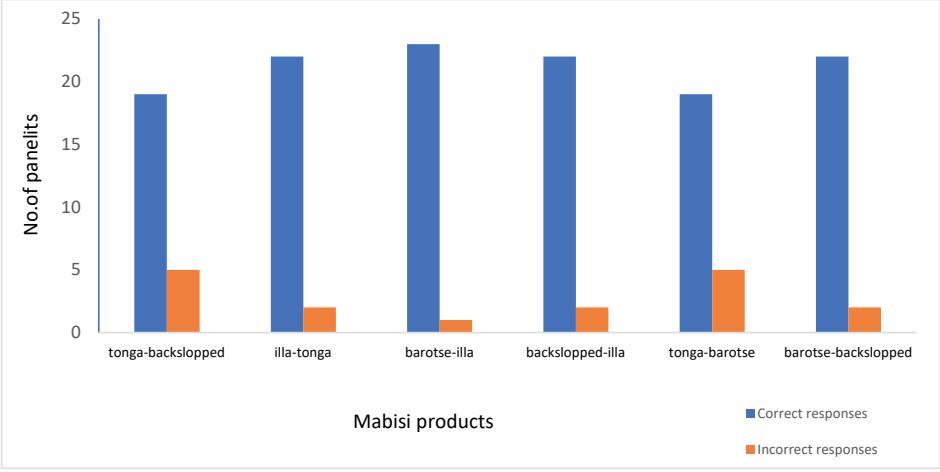

Fig. A1. A display of panelists' evaluation of products for the triangle discrimination test, showing the panel responses for the different product combinations.

Table A1. Relation between overall acceptability and hedonic attributes between mabisi variants.

	Tonga		Barotse		Backslopping		Illa	
Overall acceptance	R	p_value	R	p_value	R	p_value	R	p_value
Appearance	0,11	0,068	0,85	<0.001	0,21	<0,001	0,31	<0,001
Aroma	0,24	<0,001	0,9	<0.001	0,14	0,02	0,42	<0,001
Oral consistency	0,21	<0,001	0,91	<0.001	0,27	<0,001	0,29	<0,001
Taste	0,28	<0,001	0,92	<0.001	0,23	<0,001	0,36	<0,001
Visual consistency	0,16	<0,008	0,84	<0.001	0,16	0,007	0,25	<0,001

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CHAPTER

Odour-active aroma compounds in traditional fermented dairy products: The archetypical case of mabisi that supports food and nutrition security in Zambia

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Under review with the Current Research in Food Science journal.

Abstract

Aroma is a key sensory attribute that determines consumer preference and acceptability of foods. The aroma of fermented dairy products comprises the volatile organic compounds (VOCs) produced by the activity of fermenting microbes and the compounds originally present in unfermented raw milk. A unique combination of specific compounds detectable by human olfactory senses creates the distinct odour profile of fermented products. This study investigated the influence of different production methods on the VOCs responsible for the odour-active compounds, and the microbial communities present in mabisi, a traditional Zambian fermented dairy product. The VOCs and microbial community composition of four mabisi variants were investigated using GC-O-MS and PTR-QiTOF-MS techniques, and 16S rRNA amplicon sequencing, respectively. A panel of three assessors identified the odour-active compounds from the GC-O-MS, and the compound's quantitative aspects were obtained by the PTR-QiTOF-MS.

Twelve volatile compounds were identified as odour-active compounds during the GC-O-MS analysis. The most prominent were ketones and esters, which imparted a buttery and fruity aroma, respectively. The PTR-QiTOF-MS run identified and quantified a total of 390 m/z peaks, 55 of which were tentatively identified. 16S rRNA amplicon sequencing revealed a diverse microbial community, with *Lactococcus* species dominating. While the VOCs profiles showed significant variation in functionality among the variants, minor differences were observed in microbial composition. The study confirms that high compound concentration does not necessarily correlate with compound odour activity. Our findings offer insights into the significance of aromas and microbial ecology to support optimization strategies for upscaling traditional fermented products.

1.0 Introduction

Aroma is an essential attribute in food, particularly in traditionally fermented varieties, where it imparts distinctive characteristics highly valued by consumers. Its importance spans sensory appeal, cultural significance, quality and safety indication, product differentiation, health considerations, and formulation optimization (Boscaini et al., 2003; Sikombe et al., 2023; Sousa et al., 2022). Traditional fermentation is integral to the food culture of many low-income countries worldwide, where numerous foods rely on spontaneous fermentation with an undefined mix of microbes. Understanding the link between the microbial community and the produced aromas is key to successfully promoting these foods. However, such fermented foods have been understudied due to the traditional and informal nature of their production (Obafemi et al., 2022; Jyoti Prakash Tamang et al., 2020). Our study investigated this relationship using mabisi, a spontaneously fermented bovine milk product from Zambia as an archetypal example. Mabisi's unique flavor comes from a mix of volatile and non-volatile organic compounds (VOCs), primarily generated by the activity of lactic acid bacteria (LAB) (Schoustra et al., 2013).

Recent studies have outlined the diverse VOCs composition in mabisi, highlighting the presence of organic acids, alcohols, esters, and carbonyl compounds (Moonga et al., 2021). LAB, particularly *Lactobacillus* and *Lactococcus*, are the dominant species in mabisi (Schoustra et al., 2013). Similar findings are reported in other African milk products like amasi, nunu, and masai, albeit with regional variations (Akabanda et al., 2013; Isono et al., 1994; Osvik et al., 2013). VOCs formation results from the enzymatic activities of coexisting microorganisms that degrade the milk components (Dan et al., 2017; Smid & Kleerebezem, 2014). The nature and concentration of VOCs determine the distinct flavors that influence consumer perception and acceptability (Clark, 1998). Only a fraction of the volatiles, however,

significantly contributes to the aroma that is perceived by human olfaction (Baldovini & Chaintreau, 2020; Blank, 1996; Song & Liu, 2018).

Previous research used Headspace Solid-Phase Microextraction Gas Chromatography-Mass Spectrometry (HS-SPME-GC-MS) to profile the volatiles of different types of mabisi. In this study, we employed Gas Chromatography-Olfactometry-Mass Spectrometry (GC-O-MS) to identify odour-active aroma compounds and complemented this technique with real-time VOCs analysis using Proton Transfer Reaction-Quadrupole interface Time-of-Flight Mass Spectrometry (PTR-QiTOF-MS) to gain insights on compound concentrations (Yener et al., 2016). Our study examined four mabisi variants: backslopping, barotse, illa, and tonga mabisi, aiming to identify specific volatile compounds that influence sensory perception through olfactometry and characterize bacterial communities using 16S rRNA amplicon sequencing. We also explored the correlation between the volatile compounds and the microbial community to understand their relationship.

The findings of our study are crucial for identifying VOCs distinguishing traditional products and highlighting how unique fermentation practices contribute to aroma complexity. This knowledge is valuable to guide future efforts to optimize flavor and enhance the quality and consistency of traditionally fermented products for ultimate upscaling.

2.0 Materials and methods

2.1 Mabisi samples preparation

Four variants of mabisi namely, backslopping, barotse, illa, and tonga, were examined. Samples from the first and fourth production cycles were collected for all variants except tonga, which does not undergo production cycles. Instead, samples from tonga were collected from two different production days (Fig. 1). Samples were prepared in the kitchen at the University of Zambia's Department of Food Science and Nutrition, following previously outlined methods

(Moonga et al., 2019; Sikombe et al., 2023). Subsequently, samples were frozen (-20°C), shipped to Wageningen University (Netherlands), and stored at -80°C until further analysis. Analytical measurements were conducted in triplicate.

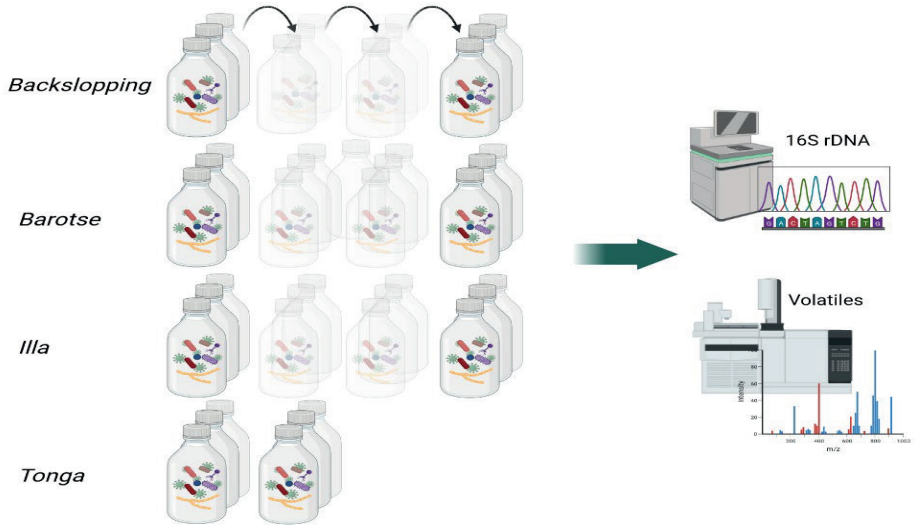

Fig. 1. Experimental design for the four mabisi variants. Tonga was prepared by two separate batches, whereas backslopping, barotse, and illa were produced by 'backslopping' the first ripe product three more times as indicated by the "shadowed" bottles. Analysis samples were taken from the first and fourth-cycle products.

2.1.1 GC-O-MS analysis

Two GC ovens, linked by a transfer line at 220°C, were employed alongside the TSQ9000 Triple Quadrupole MS (Thermo Scientific™, Waltham, USA). Detection was facilitated by the MS and a PHASER Pro GC-Olfactory Port (GL Sciences, Eindhoven, the Netherlands), both equipped with a transfer line at 220°C. Additionally, a cryo cold trap (Cryotherm, Kirchen, Germany) was integrated into the system. Compounds were desorbed from the fiber for 2 minutes and separated on a polar column (Stabilwax®-DA, 30m length, 0.25mm ID, 0.5µm df Restek, Bellefonte, USA). A Programmable Temperature Vaporizing (PTV) inlet, heated to 250°C, served as the sample inlet and operated in split mode at a 50:50 ratio for simultaneous acquisition of odour characteristics by panelists at the sniffing port and compound

identification in MS mode. The GC oven temperature began at 35°C for 5 minutes, raised to 240°C at a rate of 10°C/min, and maintained for 5 minutes. Helium was the carrier gas at a constant flow rate of 1.2 mL/min. Mass spectral data was collected over a mass-to-charge ratio (m/z) range of 33–250 in full-scan mode at 3.0030 scans/second.

Mass spectral data were analyzed using Chromoleon® 7.2.10 (Thermo Scientific™, Waltham, USA). The NIST 2014 main library was used for component matching. An alkane standard solution (Sigma-Aldrich, Darmstadt, Germany) with pentane and heptane was measured with an SPME fiber and direct injection, and the retention times were used for calculating the Linear Retention Index (LRI) using the following formula:

$$\boldsymbol{LRI = 100.\left[z + \left(\frac{tri - trz}{tr(z+1) - trz}\right)\right],}$$

where tr is the retention time; z is the previous alkane number; z+1 is the following alkane number and 'i' is the target compound.

The GC-O effluent was sniffed by a panel of three non-smoking female assessors, aged 29.7 ± 4.5 years. Each assessor evaluated two samples per day, with at least a 2.5-hour break between samples to prevent olfactory fatigue. To minimize odour interference, assessors refrained from wearing perfume and avoided strongly scented foods, such as garlic, the evening before and on the day of testing. The compounds were identified with MS and GC-O responses were recorded using synchronized audio tape during sample injection. The detected odours were described and recorded by the assessors. The odour descriptors were matched with peaks based on compound retention time and assessor response time. The responses were considered valid when at least two of the assessors were able to detect the peak. Peaks perceived by only one assessor were considered noise. Compound identification and odour descriptors were verified using the LRI and the Flavornet database (Acree & Arn, 2004).

2.1.2 PTR-QiTOF-MS analysis

Two ml samples in 250 ml beakers were agitated in a water bath at 25 ºC for 30 min. Samples were measured in a PTR-QiTOF-MS (Ionicon Analytik gmbh, Innsbrück, Austria) for 60 sec, with an acquisition rate of one spectrum/sec (m/z range 0.00 – 570). Measurements were initiated with a 5-second flushing of the PTR machine with ambient air as a blank, followed by sample measurement. The measurement was conducted in Vmode in the following ionization conditions: ion source voltage Us and Uso of 145.0 and 76.6 V, respectively, drift voltage of 999.0 V, drift temperature of 60 °C and drift pressure of 3.79 mbar corresponding to an E/N value of 134 Townsend. A mass resolution above 4000 was held throughout the run. An internal reference standard with peaks at m/z 203.943 and 330.856 was continuously injected using the PerMaScal device. The data were blank-corrected by t-tests, removing masses with no significant difference to the blank and those with a significant negative difference. This reduced m/z peaks from 534 to 390.

2.1.4 Microbial composition

Mabisi DNA extraction followed the protocol of Schoustra and colleagues (2013). DNA quality and concentration were assessed using NanoDrop™ ND-2000 and Qubit™ 4 fluorometer (Thermo Scientific, UK). Samples were sent to Novogene (UK) for V3-V4 hypervariable region 16S rRNA amplicon sequencing on Illumina NovoSeq 6000. PCR amplification used primers 341F and 806R, and amplicons were pooled, end-repaired, A-tailed, and ligated with Illumina adaptors. Paired-end reads were trimmed and filtered with fastp, FLASH, and DADA2. Chimeric sequences were removed with Vsearch. QIIME2 generated Amplicon Sequenced Variants (ASVs) using the Silva database. Phylogenetic relationships were examined with QIIME2 by aligning multiple sequences. ASV abundance was rarefied to a standard read number, and the abundance table was obtained at kingdom to genus levels.

2.2. Statistical analysis

Means and standard deviations for all data sets were calculated in Excel. All other analyses were performed in R Version 4.3.1. The VOCs data were median normalized and ANOVA was performed, followed by a post-hoc Tukey test. Principal component analysis (PCA) and Heatmaps to visualize the VOCs data were performed using the FactoMineR package (Version 2.9) and pheatmap package (Version 1.0.12) respectively. The Phyloseq package (Version 1.46) was used for microbiome data analysis, while a correlation between the VOCs and the bacterial taxa was performed using the Spearman correlation coefficient. Significance for all analyses was considered at $P < 0.05$.

3.0 Results

3.1. Volatile compounds detected by the GC-O-MS

The GC-O-MS results show variations in compound diversity and peak area percentages, indicating differences in VOCs' types and quantities (Table. S1). Twenty-six VOCs were identified, including esters, ketones, hydrocarbons, aldehydes, alcohols, carboxylic acids, hydrocarbons, and sulphur-derived compounds. While significant differences were found between the variants for most VOCs except for acetone, diacetyl, limonene, and 2-nonanone ($P < 0.05$), differences between product cycles were only detected in barotse.

The first two components of the PCA of VOCs data explained 52.8% of the total variation, revealing five distinct clusters (Fig. 2). While samples from different production cycles clustered together across the variants, barotse1 formed a separate cluster from barotse4, with the latter projecting onto the negative dimension of PC2. Barotse1, illa, and backslopping samples projected on the negative dimension of PC1 while tonga samples were separated from other variants along the positive dimension of PC1.

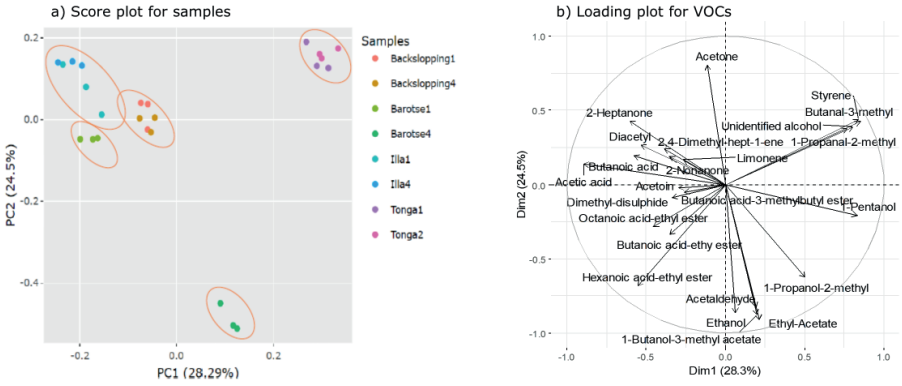

Fig. 2. PCA plot of the peak area percentages of volatiles identified by GC-O-MS in four variants of mabisi and specific compounds related to each variant. Score plots showing the clusters of the mabisi variants with each sample represented by a dot (a), the loading plots of the projection of different VOCs compounds (b).

The most dominant compounds were alcohols, particularly ethanol and 1-pentanol. Barotse4 correlated highly with 1-butanol-3-methyl acetate, ethanol, ethyl acetate, 2-methyl-1-propanol, and acetaldehyde, while barotse1 and illa samples were closely related to acetic acid, butanoic acid, and 2-nonanone. Ethanol, acetic acid, 2-nonanone, butanoic acid, octanoic acid, acetaldehyde, and ethyl acetate distinguished the samples from the two production cycles of barotse. Tonga samples were associated with 2-methyl-1-propanal, 3-methyl-butanal, styrene, and an unidentified alcohol. Acetoin, dimethyl disulfide, and ethyl butanoic acid were distinctive compounds for backslopping samples.

3.1.2 Odour-active volatile compounds detected by Olfactometry

The panel of assessors detected and described 12 volatile compounds as odour-active in mabisi. The odour-active volatile compounds and the number of panelists that could detect an odour at the sniffing port of the GC-O at a given time are presented in Fig. 3. The compounds included 2-methyl-1-propanal, 3-methyl-butanal, diacetyl, ethyl-butanoic acid, dimethyl-disulphide, ethyl-hexanoic acid, styrene, acetoin, ethyl octanoic acid, butanoic acid, and unidentified

alcohol. Barotse4 had the highest number of perceivable odours with eight odour-active volatiles, while barotse1 and tonga2 each had six compounds identified. Similarly, backslopping1 and illa1 contained four identified odour-active compounds each. Diacetyl and ethyl octanoic acid were the most common odour-active volatiles as they were detected in all four mabisi variants.

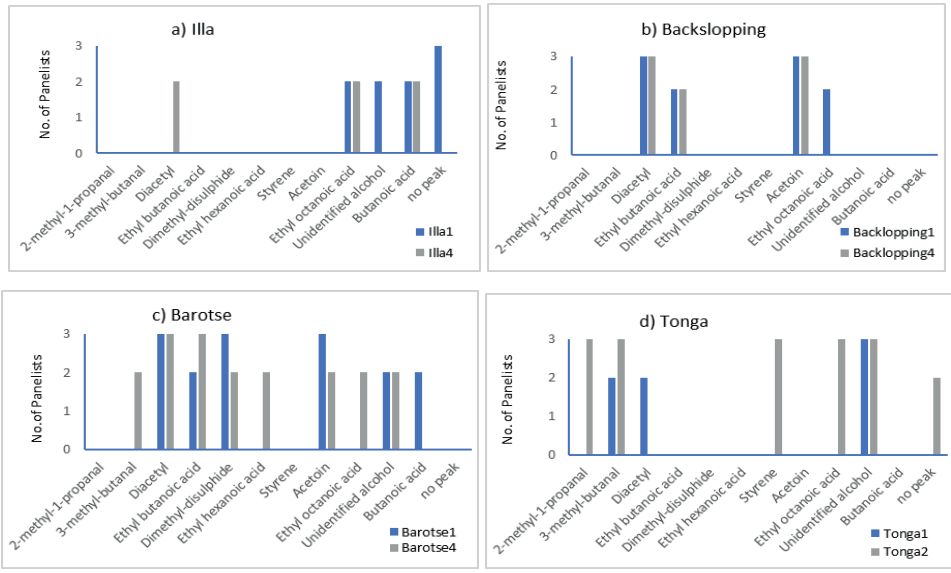

Fig.3 Sniffing bar charts of volatile compounds of (a) Illa, (b) Backslopping, (c) Barotse, and (d) Tonga obtained by gas chromatography−olfactometry (GC-O) using a panel of three assessors.

The odour-active compounds identified by olfactometry and the linear retention index on the Stabilwax®-DA column with the description of the odours by the panelists are further presented in Table 1. Diacetyl was described as sour, buttery, caramel, creamy, and fruity, while ethyl octanoic acid imparted fruity and sour aroma notes to mabisi. Like diacetyl, acetoin was described by a typical buttery caramel, creamy, sweet, and sour aroma. Interestingly, despite acetoin being detected in the MS mode in all the variants except tonga, its odour activity was only perceived in barotse and backslopping mabisi. Likewise, ethyl butanoic acid was

detected in the MS mode in all four mabisi variants but could only be perceived by olfactory senses in barotse and backslopping samples. Ethyl butanoic acid, like the other esters, contributed sweet, fruity, and fatty aroma notes to mabisi.

Table 1. Key odour-active compounds of four variants of mabisi, including their LRI as calculated on the stabilwax column, and odour descriptors by the GC-O-MS the panelists

Volatile compound	Mean RT	Mean LRI[¥]	Odour description by panelists
Propanal, 2-methyl	7,572	823,4	unpleasant, sharp, pungent, hazelnut
Butanal, 3-methyl	9,097	932,16	creamy, cocoa, nutty
Diacetyl	10,078	993,65	buttery caramel, creamy, sour, sweet, burnt milk
Butanoic acid, ethyl ester	10,98	1053,55	fruity, ripe lemon, sweet, strawberry, apple,
1-Propanol, 2-methyl	11,733	1103,07	strongly fruity, grape, sulphury
Dimethyl disulphide	11,752	1104,82	sharp, swampy, floral, vinegar
Hexanoic acid, ethyl ester	14,032	1248,8	banana, weakly fruity, sour
Styrene	14,616	1288,31	soapy, floral, buckwheat, herbal, mushroom
Acetoin	14,981	1313,94	buttery, creamy, sour, caramel
Octanoic acid, ethyl ester	16,83	1450,82	Sharp, fruity, sour
Unidentified alcohol	17,55	1505,55	cooked meat, unpleasant, stuffy, mushroom, rancid, putrid, sour, pungent, slightly sulphury, creamy, salty caramel, toasted almonds
No peak*	n.a	n.a	sharp, cinnamon, rancid, old smoke
Butanoic acid	19,293	1645,01	rancid, cheese, stale

[¥] *LRI, linear retention index of the compounds on a Stabilwax® DA column*
** No peak was detected by the GC-MS, but the panelists perceived and described an odour through olfactometry.*

Butanoic acid was the only carboxylic acid with odour activity in mabisi, imparting rancid, cheese, stale, and weakly cardboardy aroma perceived in barotse1 and illa4. Two branched-chain aldehydes, 3-methyl-butanal and 2-methyl-1-propanal, were also identified as odour-active volatiles in barotse and tonga mabisi. These aldehydes contributed an overall nutty flavor, despite apparent differences in their characteristic aromas. To illustrate, while a creamy aroma was attributed to 3-methyl-butanal, 2-methyl-1-propanal was characterized by a pungent and somewhat unpleasant aroma.

Certain compounds were exclusively found in some mabisi variants but not others. For instance, ethyl-hexanoic acid was only perceived in barotse4 while dimethyl-disulphide, described as having a "sharp, swampy, and floral" aroma, was present in both cycles of the barotse samples. Similarly, styrene, characterized by a soapy, floral, buckwheat, herbal, and mushroom-like aroma, was unique to tonga2 samples. Additionally, an unidentified alcohol contributed to a range of aromas in both barotse and tonga samples, including cooked meat, sweaty, rancid, putrid, sour, pungent, sharp, slightly sulphury, salt caramel, and toasted almonds. Tonga2 and illa1 had an odour referred to as ‘no peak’ as it was perceived by the panelists and not detected in MS mode. The ‘no peak’ was described as having flower, sharp, cinnamon, rancid, and old smoke aroma.

3.2 Volatile compounds detected by the PTR-QiTOF-MS

We used the PTR-QiTOF-MS to quantify headspace compounds and complement the GC-O-MS findings to gain insights into VOC's quantitative aspects. As expected, the PTR-QiTOF-MS results revealed a more complex volatile profile than the GC-O-MS. A heatmap depicting the intensity (i.e., concentrations in ppbv) for each of the mass peaks, (Fig. 4) and cluster analysis (Fig. S1) demonstrated the ability of PTR-QiTOF-MS to detect the variability between the cycles of barotse, backslopping, and illa. While backslopping1 had higher concentrations and more diverse range of volatiles than backslopping4, the fourth cycles for illa and barotse exhibited greater diversity and concentrations compared to their first cycles. Low volatile concentrations were observed in all tonga samples. Barotse4 had the highest volatile concentrations and variations. Peaks that significantly differentiated samples and those unique to specific samples were identified (Table. S1). Consistent with heatmap results, VOCs concentrations in backslopping decreased over cycles, this could be further investigated to optimize the quality of backslopping mabisi.

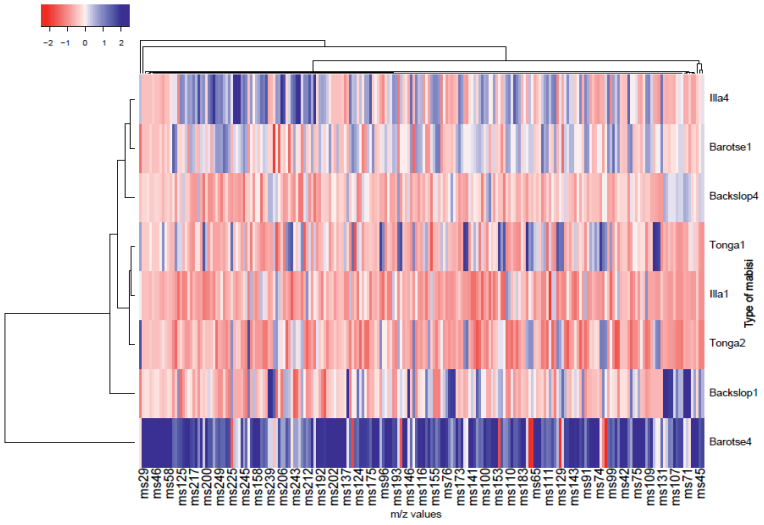

Fig. 4. Heatmap matrix of the normalized data of VOCs of the mabisi variants obtained by PTR-QiTOF-MS. The x-axis represents mass peak/charge ratio and the y-axis represents the mabisi variants. Intense blue color represents a high concentration while intense red color represents a low concentration.

3.1.4.2 Tentative identification of m/z peaks to complement the GC-O-MS findings

Fifty-five m/z were tentatively identified by comparing PTR-QiTOF-MS masses with the NIST library and literature (Table. S2). A mass deviation of 0.001 was allowed as a threshold. Since multiple compounds can have the same or similar mass, a peak may correspond to more than one compound at the same time (Aprea et al., 2015; Bottiroli et al., 2020; Soukoulis et al., 2010; Yener et al., 2016).

The compounds with mass peak at m/z 89.0602 significantly distinguished the backslopping samples. These could be tentatively identified as acetoin, ethyl acetate, or butanoic acid as they have a similar molecular weight. However, acetoin was particularly distinct when comparing the PTR-QiTOF-MS and the GC-O-MS results. It was also perceived by olfactometry as an odour-active volatile in backslopping. Therefore, the peak at m/z 89.0602 is most likely acetoin. Additionally, the mass peak at m/z 87.0440 was identified as diacetyl and was

significantly higher in backslopping1, although it expressed odour activity in all mabisi variants.

The compounds with mass peaks at m/z 45.0336 and m/z 47.0492 were tentatively identified as acetaldehyde and ethanol. These compounds had the highest peaks during the PTR-QiTOF-MS run, and their presence was most prominent in barotse4. While acetaldehyde did not have the highest peak during GC-O-MS analysis, ethanol in barotse4 was still the highest peak in the GC-O-MS analysis.

Other peaks, among them, m/z 145.1232 and m/z 117.0911 tentatively identified as ethyl hexanoic acid (which could also be octanoic acid) and ethyl butanoic acid (which could be hexanoic acid) significantly differentiated barotse4 from the other mabisi products. These compounds were also identified as odour-active volatiles. In the illa samples, the mass peak at m/z 117.0911 and tentatively identified as butanoic acid ethyl was distinctive for illa4. A compound with mass peak at m/z 87.0801 and tentatively identified as butanal-3-methyl was distinctive for tonga1. However, this peak could also represent 2-pentanone or pentanal. But since butanal-3-methyl was distinctive for tonga and an odour-active volatile for both tonga samples, it is likely that this particular mass represents butanal-3-methyl.

Only two mass peaks had concentrations above 1 ppm, specifically m/z 47.0492 (ethanol) and m/z 45.0336 (acetaldehyde), both of which were associated with barotse4. The other peaks, including m/z 72.0516, 87.044, 87.0801, 94.0727, 105.0708, 117.0911, 145.1232, and 173.1521 (propanal-2-methyl, diacetyl, butanal-3-methyl, dimethyl-disulphide, styrene, ethyl butanoic acid, ethyl hexanoic acid, and ethyl octanoic acid, respectively), had concentrations below 10 ppbv. The mass peak at m/z 89.0602, tentatively identified as acetoin/butanoic acid, had higher concentrations ranging between 7.3 and 151 ppbv across the different mabisi variants.

3.2 Taxonomic identification of the bacterial communities in mabisi

The 16S rRNA amplicon sequencing results showed a diverse community of bacteria dominated by the phylum Pseudomonadota and Bacillota. The bacteria were taxonomically classified up to the genus level. Bacterial taxa were visualized using bar plots and a total of 14 genera were identified, while the species with an abundance below 1% were assigned to others (Fig. 5).

Fig.5. Taxonomic identification of the bacterial communities in the four mabisi products showing the relative abundance at the genus level. The different colors represent the most abundant genera of the taxa units.

In general, species of the genera *Lactococcus*, *Aeromonas, Acetobacter, Lactobacillus, Klebsiella, Escherichia-shigella, Streptococcus, Acinetobacter,* and *Enterococcus*, in order of relative abundance, made the top ten species in the samples. Among the bacterial genera identified, *Lactococcus* was dominant in all the samples with relative abundances ranging from 20 – 90%, stressing the importance of this species as an acid producer in milk fermentation.

After *Lactococcus*, members of the genus *Lactobacillus* dominated backslopping1, barotse1 and 4, and illa1 whereas *Aeromonas* was the most abundant genus in backslopping4. *Aeromonas* also dominated the tonga and illa4 samples. Some species of *Bifidobacterium* were observed in barotse and illa samples.

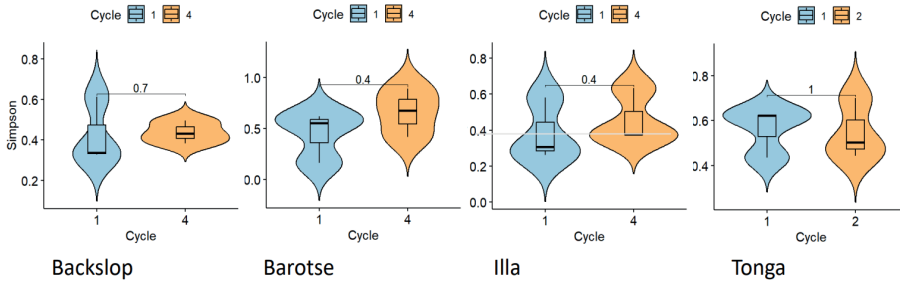

Fig.6. Alpha diversity-Simpson indices showing variation in the bacterial community diversity between cycles of the mabisi products. $p > 0.05$

Alpha diversity analysis, as determined by the Simpson index, revealed that barotse4 and tonga1 exhibited the highest microbial diversity, whereas backslopping1 and illa1 displayed the lowest diversity (Fig.6). In general, the microbial diversity for the first cycles across all the products except for tonga mabisi was lower than their subsequent cycles. The variation in microbial diversity between cycles was slightly more pronounced in the barotse compared to the other variants. However, no significant difference in diversity was evident between the respective cycles of the variants ($p > 0.05$). Regarding the Observed alpha diversity, illa1, and tonga1 exhibited a greater microbial community richness (Fig.S2). Interestingly, only barotse variants displayed increased species richness in subsequent cycles, with the "Observed" alpha diversity. The other variants' species richness was apparent in the first cycle samples. Similarly, no significant differences were observed across the cycles of the mabisi variants in terms of "Observed" alpha diversity ($p > 0.05$).

Despite the significant differences between products regarding their volatile compounds, a Bray-Curtis dissimilarity clustering identified minor variations in the microbial communities of the different products (Fig. 7). Barotse samples appeared to be isolated and mostly cluster together, while the other three variants seemed to overlap with each other. Barotse may possess a subtly distinct bacterial community compared to the other variants.

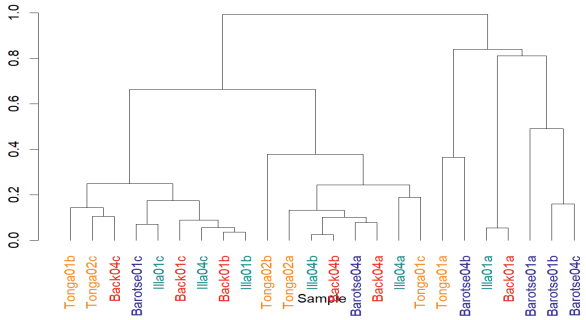

Fig.7. Dendrogram cluster representation of the genus level community dissimilarity for the mabisi variants as measured by Bray-Curtis.

3.3 Correlation between odour-active aroma compounds and bacterial genera

The potential relationship between the aroma compounds and the bacterial genera in the mabisi samples is illustrated in a heatmap (Fig. 8). Focus was given to the odour-active compounds and correlations were observed between bacterial genera and the volatile compounds. *Acetobacter, Enterobacter, Pseudomonas, Aeromonas,* and *Lactobacillus* seemed to correlate with the highest number of odour-active compounds. *Lactococcus* exhibited a limited positive correlation with diacetyl and acetoin. The esters of octanoic acid, hexanoic acid, and butanoic acid show a positive association with *Lactobacillus* and *Streptococcus. Lactobacillus* further correlated positively with butanoic acid, dimethyl-disulphide, and acetoin.Other odour-active volatiles such as 1-propanal-2-methyl, styrene, butanal-3-methyl, and the unidentified alcohol showed a positive correlation with *Pseudomonas* and the unassigned genus. In addition, these

volatiles, except styrene, were positively correlated with *Acetobacter*, while styrene was associated with *Aeromonas.*

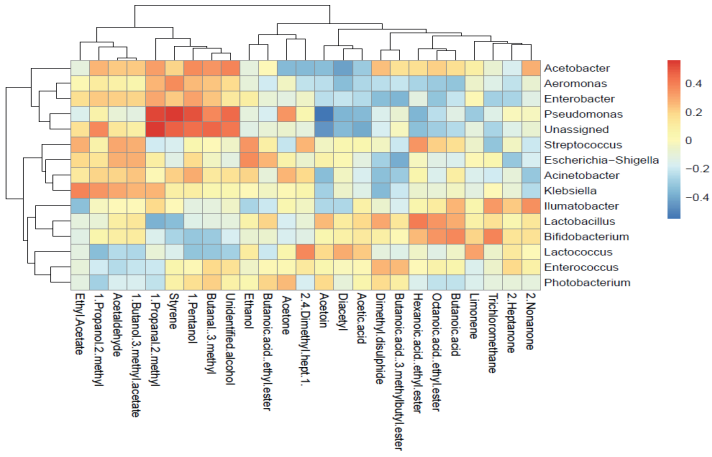

Fig. 8. A correlation heatmap and dendrogram matrix displaying the relationship between VOCs (GC-O-MS) and dominant bacterial genera using the Spearman correlation coefficient. Deep red color indicates a perfect positive correlation, while deep blue indicates a perfect negative correlation.

4.0 Discussion

Our study aimed to analyze the VOCs in mabisi and identify specific compounds that contribute to its aroma using olfactometry. Additionally, the microbial community was characterized using 16S rRNA amplicon sequencing.

Most of the volatiles identified in this study have been reported in other fermented dairy products such as kefir and yogurt, including previous studies on mabisi (Benkerroum & Tamime, 2004; Beshkova et al., 2003; Irigoyen et al., 2012; Moonga et al., 2021; Settachaimongkon et al., 2014). However, the compound 2,4-dimethyl-1-heptene is relatively uncommon in fermented milk products (Cheng, 2010; Jiang et al., 2020). It is a possible contaminant from the plastic containers used during fermentation as it is reportedly a degradation product of polypropylene (Soják et al., 2007). While among the identified odour-

active compounds, dimethyl-disulphide is quite unusual in mabisi. However, it is common in other fermented dairy products, such as yogurt and cheese, where it imparts a sulphur aroma and its presence is linked to the microbial breakdown of methionine (Brattoli et al., 2013; Moonga et al., 2021; Ott et al., 1999; Smit et al., 2005; Yvon & Rijnen, 2001).

The variants of mabisi exhibited unique combinations of volatile components, contributing to their distinct odour-active profile. The fact that only 12 of the detected 26 volatiles were perceived by olfactometry shows that not all VOCs in the headspace contribute to the perceived aromas of the product. This may be attributed to the fact that despite a response in the MS mode, concentrations may be relatively low for the odour detection threshold and, therefore could not be perceived by the nose. Ethanol and acetaldehyde perfectly depict this, despite their high concentrations, these compounds remained imperceptible to the nose. Whereas, the presence of ethyl octanoic acid and butanal-3-methyl, with low concentrations, could be perceived as odour-active volatiles. Conversely, some compounds could be perceived at the sniffing port but not detected in the MS mode. This could mean a low odour detection threshold with concentrations below the GC-MS detection limit. Similar observations have been reported in other studies (Pang et al., 2012), but this could also be potential thermal artifacts from the GC inlet or column, especially at later retention times (Baldovini & Chaintreau, 2020). Thus, illustrating that the sensory relevance of a volatile compound depends on the headspace concentrations and compound odour detection threshold. Therefore, just because a compound is abundant or prominent in the MS response does not mean it will be perceived as odour-active. Thus, olfactometry provides insights into volatiles of sensory significance that contribute to the overall aromas of a product.

Compared with the other variants, barotse had a more diverse and intense odour-active profile and this aligns with earlier findings on the sensory evaluation of mabisi where barotse was characterized as having a more intense odour than the other variants (Sikombe et al., 2023). Of

the odour-active volatiles diacetyl and ethyl octanoic acid were the most common odour-active compounds, a combination that contributed buttery, caramel, creamy, fruity, and fatty aroma notes to mabisi. Diacetyl is a common volatile in fermented dairy products such as yogurt and buttermilk. Together with acetoin, diacetyl forms the principal metabolic products of the microbial activity on the milk components (Erkus et al., 2014; Friedrich & Acree, 1998). They are associated with certain mesophilic bacteria such as Lactococcus spp. and are produced through citrate metabolism (Curioni & Bosset, 2002; McSweeney & Sousa, 2000). It is not surprising that they are odour-active compounds in mabisi as Lactococcus spp. was the predominant species across all the mabisi variants, stressing the importance of this species in developing the characteristic aromas of mabisi. The dominance of Lactococcus spp. is expected as mabisi production is conducted at ambient temperatures of 22-33 ºC providing optimum growth conditions for this species (Batt, 2014). A positive correlation was observed between the odour-active ester compounds and Lactobacillus spp. and Streptococcus spp. this is expected as the lipolytic nature of some species of LAB, through enzyme production such as lipase and esterase, is attributed to their association with ester formation (McSweeney & Sousa, 2000). In dairy products, lipolysis may result from the indigenous milk lipases or microbial lipase activity. Esters are produced from the esterification of fatty acids and alcohols derived from microbial fermentation of lactose or the breakdown of milk fats. However, the role of yeasts in ester production and volatile formation, in general, cannot be overlooked, as yeasts are also present in mabisi despite their low abundance (Schoustra et al., 2013). Lactobacillus spp. was further correlated with butanoic acid, an important flavor component of fermented dairy products and also a common product of the lipolysis of milk fat or the degradation of specific branched-chain amino acids such as leucine, isoleucine, and valine (Aiello et al., 2023; Curioni & Bosset, 2002).

A nutty flavor was contributed by the two branched-chain aldehydes 3-methyl-butanal and 2-methyl-1-propanal. Excessive levels of 2-methyl-1-propanal have been associated with off-flavors in certain fermented dairy products. However, when present in balanced proportions with other volatile compounds, it can positively contribute to the overall aroma profile of the product (Yvon & Rijnen, 2001). These methyl aldehydes are a product of the metabolism of branched-chain amino acids involving certain wild strains of L. lactis, and have been identified as key odourants in artisanal fermented milk products like cheese (Ayad et al., 1999).

The microbial communities of the tonga and barotse variants were more diverse than the other variants, supporting the findings of Moonga et al. (Moonga et al., 2020). Additionally, despite significant differences in the volatile compounds among the variants, their microbial communities did not exhibit significant differences. This lack of distinction is likely due to the genus-level identification being insufficient to reveal these differences; we presume that species-level or lower taxonomical levels, such as genetic lineage, could provide more detailed differences. Unfortunately, this is the limitation of 16S rRNA profiling methods which lack the ability to provide resolution at species and, subsequently, strain level. This lack hinders further understanding of the subtle shifts in microbial composition along with changes in the functional capabilities of the microbiome. Additionally, maintaining similar conditions during the production of the different products, including temperature and exposure to the same environmental microbes, may have minimized the differences in community composition.

The ability of microbes to produce aroma compounds through proteolysis, lipolysis, or citrate metabolism is strain-dependent. The community of microbes associated with the volatile compounds in this study may have been a narrow representation of key players in the overall aroma production of mabisi. Other groups of microbes, such as yeasts, are also believed to play a role in traditional dairy fermentation and thus aroma formation of these products (Yvon & Rijnen, 2001).

Volatile compounds in fermented products are produced via complex metabolic reactions involving enzymatic activities on milk components such as carbohydrates, proteins, and lipids (Smid & Kleerebezem, 2014). A diverse community of lactic acid bacteria drives these metabolic processes and is responsible for the various aromas that are produced. However, the ability of these microorganisms to produce aroma compounds through proteolysis, lipolysis, or citrate metabolism is strain-dependent. The community of microorganisms associated with the volatile compounds in this study may have been a narrow representation of key players in the overall aroma production of mabisi. Other groups of microorganisms, such as yeasts, are also believed to play a role in traditional dairy fermentation and thus aroma formation of these products (Yvon & Rijnen, 2001). Identifying odour-active compounds in traditional fermented products and the description of these odours is vital for re-creating these distinct aroma notes, particularly through techniques like the aroma recombination approach (Ott et al., 1999). By understanding the specific odour-active compounds and their contributions to the overall aroma, researchers and producers can accurately replicate the unique and desirable aroma characteristics of different variants of traditional fermented products. This could enhance the optimization of the sensory qualities, thereby helping to preserve the traditional flavors for broader consumer appeal.

5.0 Conclusion

The GC-O-MS and PTR-TOF-MS analyses demonstrated perceivable differences between the four variants of mabisi regarding their odour-active properties and overall volatile profiles, while the composition of the microbial community at the genus level showed minor variations. The study further demonstrates that high detector response or high concentrations do not automatically correspond to compound odour activity as seen by the selectivity of the GC-O-MS towards low odour threshold compounds. This knowledge has far-reaching implications for traditionally fermented products. It does not only provides valuable insights for tailoring

flavor profiles to align with consumer preferences but could also be useful for assessing product quality and detecting incidences of contamination or spoilage in fermented foods.

Acknowledgments

We would like to express our gratitude to the Integrated Research Fund (INREF) of Wageningen University and Research which funded the activities of this research. We would further like to thank the laboratory scientists and technicians from WUR and UNZA for their assistance.

Supplementary material

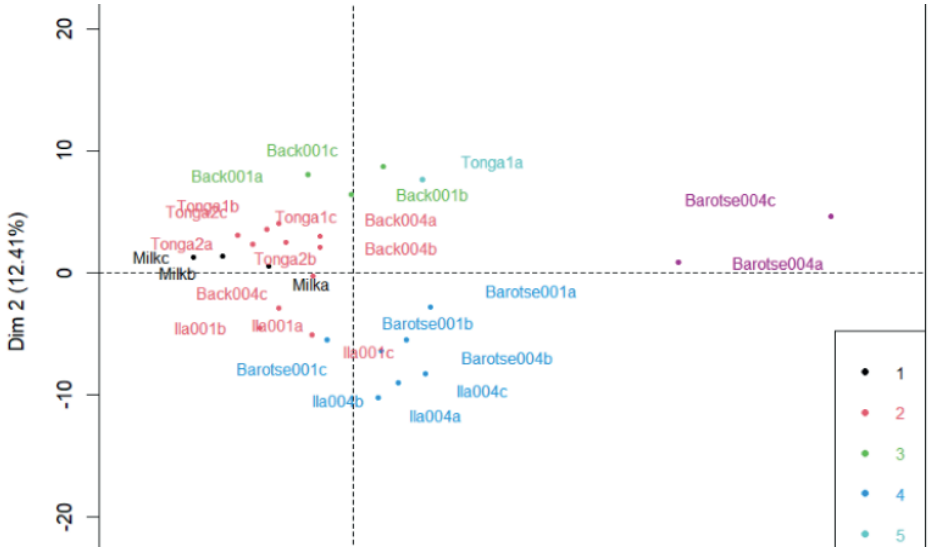

Fig. S1. Clusters of the mabisi variants and their respective production cycles based on normalized data (concentrations (ppbv) of the VOCs) obtained by PTR-QiTOF-MS measurements. The clustering was performed with HCPC function in R on PCA results. Color variations represent the six different clusters that were formed.

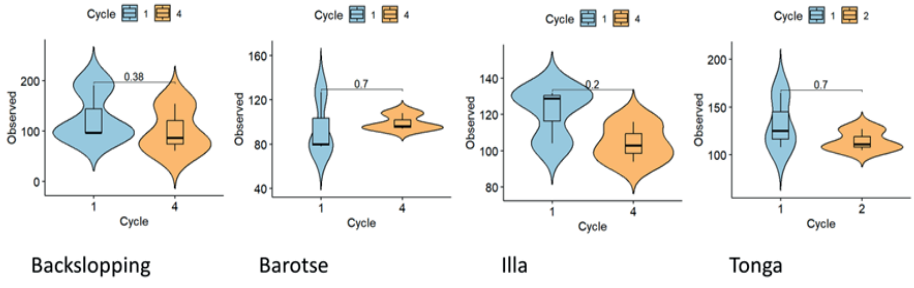

Fig. S2. Alpha diversity-Observed, showing the variation in the species richness of bacterial community between cycles of the mabisi products. $p > 0.05$

Table S2. Means and standard deviations of peak area percentages of volatiles identified by GC-MS during GC-O-MS measurements in the different types of mabisi. Relative peak areas were obtained by calculating a percentage of each peak of the total area of all peaks in a sample. Significant differences between samples are indicated by different letters (Kruskal-Wallis rank sum test, Bonferroni, α=0.05). Additionally, the linear retention index (LRI) of the compounds on a Stabilwax® DA column are given.

Compound	LRI	Peak area percentages (Mean ± SD)								Identification
		Illa1	Illa4	Backlopping1	Backlopping4	Barotse1	Barotse4	Tonga1	Tonga2	
Acetaldehyde**	703.44	0.00	0.00	0.00	0.00	0.00	0.90 ± 0.55^{a}	0.00	0.00	LRI, MS
1-Propanal-2-methyl**	823.40	0.00	0.00	0.00	0.00	0.00	0.00	1.53 ± 1.3^{a}	2.93 ± 0.7^{a}	LRI, MS, GCO
Acetone^{ns}	826.43	4.57 ± 1.9	4.26 ± 1.4	3.49 ± 0.3	3.59 ± 1.0	1.85 ± 0.8	0.00	4.52 ± 1.5	3.32 ± 0.4	LRI, MS
2,4-Dimethyl-hept-1-ene*	884.84	13.3 ± 10.7^{a}	7.81 ± 1.7^{a}	1.64 ± 2.8^{ab}	2.59 ± 2.4^{ab}	1.91 ± 0.6^{ab}	0.57 ± 0.6^{ab}	2.82 ± 1.0^{ab}	1.84 ± 1.6^{ab}	LRI, MS
Ethyl-Acetate*	898.99	0.50 ± 0.9^{ab}	0.00	0.00	1.19 ± 1.2^{ab}	0.00	7.95 ± 0.8^{a}	0.59 ± 1.0^{ab}	0.00	LRI, MS
Butanal-3-methyl**	932.16	0.00	0.00	0.00	0.57 ± 1.0^{cd}	3.6 ± 0.7^{ab}	1.06 ± 0.2^{bc}	28.46 ± 3.1^{a}	25.8 ± 1.7^{a}	LRI, MS,GCO
Ethanol**	941.81	18.26 ± 15^{ab}	5.94 ± 0.2^{de}	16.55 ± 4.2^{ab}	19.28 ± 2.7^{a}	5.8 ± 0.8^{de}	43.9 ± 2.3^{a}	6.75 ± 0.3^{cd}	7.13 ± 1.0^{bc}	LRI, MS
Diacetyl^{ns}	993.65	2.05 ± 1.8	2.93 ± 1.5	2.30± 0.8	2.33± 0.6	0.85± 0.8	0.26± 0.2	1.10± 1.0	0.00	LRI, MS, GCO
Trichloromethane^{ns}	1037.62	12.7 ± 14	15.92 ±12.2	13.78 ± 8.4	8.21 ± 6.2	5.58 ± 9.7	0.15 ± 0.2	1.55 ± 1.3	10.60 ± 9.68	LRI, MS
Butanoic acid-ethy ester*	1053.55	1.67 ± 0.34^{abc}	1.78 ± 1.87^{ab}	2.97 ± 1.53^{abc}	3.6 ± 1.46^{ab}	5.12 ± 0.74^{a}	2.86 ± 0.49^{ab}	1.64 ± 0.36^{abc}	1.33 ± 0.63^{bc}	LRI, MS, GCO
1-Propanol-2-methyl**	1103.07	0.00	0.00	0.32 ± 0.56^{ab}	0.00	0.00	2.2 ± 0.26^{a}	1.31 ± 1.13^{ab}	0.24 ± 0.41^{ab}	LRI, MS
Dimethyl-disulphide**	1104.82	0.00	0.00	0.00	0.00	2.92 ± 0.35^{a}	0.00	0.00	0.00	LRI, MS,GCO
1-Butanol-3-methyl acetate **	1141.05	0.00	0.00	0.00	0.00	0.00	0.56 ± 0.23^{a}	0.00	0.00	LRI, MS
2-Heptanone**	1202.68	6.09 ± 2.39^{ab}	7.7 ± 2.67^{a}	0.66 ± 1.15^{cd}	0.84 ± 0.75^{cd}	3.30 ± 0.30^{ab}	0.00	1.78 ± 0.35^{bcd}	1.22 ± 1.13^{cd}	LRI, MS
1-Pentanol**	1213.462	2.86 ± 2.55^{de}	3.36 ± 1.69^{de}	8.28 ± 4.04^{cd}	10.8 ± 4.46^{bc}	13.3 ± 2.34^{bc}	17.6 ± 0.14^{ab}	19.8 ± 1.62^{a}	20.1 ± 1.62^{a}	LRI, MS
Limonene^{ns}	1224.10	4.98 ± 5.96	15.5± 26.8	4.77 ± 5.02	0.62 ± 1.07	0.32 ± 0.56	0.86 ± 0.25	0.73 ± 1.26	0.81 ± 1.41	LRI, MS
Hexanoic acid-ethyl ester**	1248.80	7.68 ± 1.73^{abc}	5.97 ± 0.62^{bcd}	4.41 ± 0.81^{de}	4.59 ± 0.72^{cd}	9.05 ± 0.53^{ab}	10.03 ± 0.48^{a}	2.54 ± 0.94^{ef}	2.12 ± 0.15^{ef}	LRI, MS, GCO
Butanoic acid-3-methylbutyl ester**	1281.09	0.00	0.00	0.00	0.00	2.02 ± 0.28^{a}	0.00	0.28 ± 0.48^{b}	0.00	LRI, MS
Styrene**	1288.31	0.00	0.00	0.00	0.00	0.00	0.00	1.71 ± 0.23^{a}	1.33 ± 0.49^{a}	LRI, MS, GCO
Acetoin**	1313.94	1.45 ± 0.48^{c}	2.55 ± 1.25^{bc}	29.9 ± 2.55^{a}	30.6 ± 3.9^{a}	8.33 ± 1.58^{ab}	1.65 ± 0.08^{c}	0.00	0.00	LRI, MS, GCO
2-Nonanone^{ns}	1313.87	0.47 ± 0.81	0.95 ± 1.65	0.48 ± 0.84	0.00	0.97 ± 0.12	0.00	0.00	0.34 ± 0.59	LRI, MS
Octanoic acid-ethyl ester**	1450.82	2.76 ± 0.45^{ab}	1.23 ± 1.17^{bc}	0.11 ± 0.2^{c}	0.86 ± 0.76^{bc}	9.87 ± 1.01^{a}	2.75 ± 0.35^{ab}	0.00	0.23 ± 0.39^{c}	LRI, MS, GCO
Acetic acid**	1469.55	11.5 ± 4.26^{ab}	18.9 ± 4.70^{a}	10.36 ± 2.04^{bc}	10.27 ± 1.23^{bc}	13.41 ± 1.42^{ab}	5.07 ± 0.47^{cd}	2.78 ± 0.33^{de}	1.53 ± 1.39^{de}	LRI, MS
Unidentified alcohol**	1505.55	0.00	0.00	0.00	0.00	7.28 ± 0.6^{b}	1.28 ± 0.12^{b}	19.7 ± 0.57^{a}	19.1 ± 1.98^{a}	LRI, MS, GCO
Butanoic acid**	1645.01	9.12 ± 7.21^{a}	5.22 ± 1.56^{ab}	0.00	0.00	4.49 ± 1.05^{ab}	0.34 ± 0.3^{bc}	0.43 ± 0.74^{c}	0.00	LRI, MS, GCO

^{ns} *p-value > 0.05* **p-value ≤ 0.05* ***p-value ≤ 0.01* ****p-value ≤ 0.001*

Table S2. Tentatively identified m/z peaks, measured by PTR-QiTOF-MS, that differentiated the mabisi products significantly and were significant in describing specific samples.

Measured m/z	Theoret. m/z	Sum formula	Tentative Identification	Chemical class	Conc. (ppb) Mean ± SD
Backslopping001					
33.0335***	33.033	CH_4OH^+	Methanol(Yener et al., 2016)	Alcohols	61.48 ± 3.57
69.0335***	69.034	$C_4H_4OH^+$	Furan(Yener et al., 2016)	Furan	0.22 ± 0.02
71.0495***	71.049	$C_4H_6OH^+$	Butenal(Yener et al., 2016)	Aldehydes	135.05 ± 22.92
87.044***	87.0441	$C_4H_6O_2H^+$	Diacetyl(Bottiroli et al., 2020; Soukoulis et al., 2010; Yener et al., 2016)	Ketone	8.67 ± 1.19
89.0602***	89.0604	$C_4H_8O_2H^+$	Acetoin/Ethyl acetate/Butanoic acid(Bottiroli et al., 2020)	Ketones/Esters/Acids	151.47 ± 24.32
91.0592*	91.058	$C_4H_{10}SH^+$	Diethylsulphide/Butanethiol (fragment)(Bottiroli et al., 2020; Yener et al., 2016)	Sulphur compounds	1.34 ± 0.27
93.0695*	93.037	$C_7H_8H^+$	Toluene(Bottiroli et al., 2020; Yener et al., 2016)	Aromatic hydrocarbons	1.02 ± 0.09
Backslopping004					
71.0495***	71.049	$C_4H_6OH^+$	Butenal(Yener et al., 2016)	Aldehydes	59.77 ± 16.46
89.0602**	89.0604	$C_4H_8O_2H^+$	Acetoin/Ethyl acetate/Butanoic acid(Bottiroli et al., 2020)	Ketones/Esters/Acids	69.33 ± 17.58
Barotse001					
60.0216***	60.0212	$C_2H_3O_2H^+$	Acetate(Patiny & Borel, 2013)	Esters	0.84 ± 0.22
81.0697**	81.070	$C_6H_8H^+$	Cyclohexadiene(Yener et al., 2016)	Terpene fragment	1.45 ± 0.05
95.0148***	95.016	$C_2H_6O_2SH^+$	Dimethyl sulfone (Methylsulfonylmethane)(Yener et al., 2016)	Sulphur compouds	0.91 ± 0.49
99.0806***	99.080	$C_6H_{10}OH^+$	Hexenal/Methylpentenone(Yener et al., 2016)	Aldehydes/Ketones	1.01 ± 0.12
109.1011**	109.101	$C_8H_{12}H^+$	Cyclooctadiene(Yener et al., 2016)	Hydrocarbons	1.96 ± 0.18
110.1045**	111.044	$C_6H_6O_2H^+$	Acetyl furan(Yener et al., 2016)	Furans	0.18 ± 0.02
117.0911***	117.0917/ 117.0913	$C_6H_{12}O_2H^+$	Ethyl butanoic acid/Hexanoic acid(Bottiroli et al., 2020)	Esters/Acids	4.53 ± 0.74
123.0461*	123.0811	$C_8H_{10}OH^+$	Phenylethyl Alcohol	Alcohols	0.42 ± 0.04

127.1119***	127.1488	$C_9H_{18}H^+$	2,4-Dimethylhept-1-ene	Alkenes	1.21 ± 0.08
137.1331*	137.133	$C_{10}H_{16}H^+$	Various monoterpenes(Yener et al., 2016)	Terpenes	1.01 ± 0.03
201.1764**	201.1856	$C_{12}H_{24}O_2H^+$	Decanoic acid, ethyl ester	Esters	0.05 ± 0.01
Barotse004					
43.054***			Alkyl fragment(Bottiroli et al., 2020)		114.99 ± 76.37
44.0209***			Acetic acid fragment(Aprea et al., 2015)	Acid fragment	16.68 ± 8.93
45.0336***	45.034	$C_2H_4OH^+$	Acetaldehyde(Yener et al., 2016)	Aldehydes	1096.83 ± 815.01
47.0492***	47.049	$C_2H_5OH^+$	Ethanol(Yener et al., 2016)	Alcohols	2331.85 ± 1853.20
57.0699***			Pentanoic acid fragment(Aprea et al., 2015)	Acid fragment	71.98 ± 51.42
60.0216***	60.0212	$C_2H_3O_2H^+$	Acetate(Patiny & Borel, 2013)	Esters	0.86 ± 0.32
61.0287***	61.028	$C_2H_4O_2H^+$	Acetic acid(Yener et al., 2016)	Acids	638.84 ± 307.61
69.0335*	69.034	$C_4H_4OH^+$	Furan(Yener et al., 2016)	Furan	0.21 ± 0.02
71.0855***			Butanoic acid fragment/Pentanoic acid fragment(Aprea et al., 2015)	Acid fragments	37.05 ± 28.35
73.0291*	73.0290	$C_3H_4O_2H^+$	2-Propenoic acid(Patiny & Borel, 2013)	Acids	0.87 ± 0.20
73.0646***	73.065	$C_4H_8OH^+$	Methylpropanal/2-Butanone/Butanal(Bottiroli et al., 2020; Yener et al., 2016)	Aldehydes/Ketones	28.94 ± 16.02
75.0437***	75.044	$C_3H_6O_2H^+$	Propionic acid(Aprea et al., 2015; Yener et al., 2016)	Acids	2.70 ± 0.83
75.0803***	75.0811	$C_4H_{10}OH^+$	1-Propanol,2-methyl	Alcohols	3.18 ± 1.90
81.0697***	81.070	$C_6H_8H^+$	Cyclohexadiene(Yener et al., 2016)	Terpene fragment	1.58 ± 0.11
85.0647**	85.065	$C_5H_8OH^+$	Pentenal/Pentenone(Yener et al., 2016)	Aldehydes/Ketones	0.91 ± 0.46
85.1008*	85.1018	$C_6H_{12}H^+$	Cyclohexane(Patiny & Borel, 2013)	Alkanes	0.50 ± 0.12
87.044**	87.0441	$C_4H_6O_2H^+$	Diacetyl(Bottiroli et al., 2020; Soukoulis et al., 2010; Yener et al., 2016)	Ketone	6.69 ± 4.14
91.0592***	91.058	$C_4H_{10}SH^+$	Diethylsulphide/Butanethiol (fragment)(Yener et al., 2016)	Sulphur compounds	1.68 ± 0.92
93.0366***	93.037	$C_7H_8H^+$	Toluene(Bottiroli et al., 2020; Yener et al., 2016)	Aromatic hydrocarbons	2.08 ± 0.30

97.0286**	97.028	$C_5H_4O2H^+$	Furfural(Yener et al., 2016)	Aldehydes	0.22 ± 0.02
97.0639***	97.065	$C_6H_8OH^+$	Hexadienal/Ethylfuran(Bottiroli et al., 2020; Yener et al., 2016)	Aldehydes/Furans	0.38 ± 0.15
97.101*			Heptanal fragment		1.08 ± 0.35
99.0806***	99.080	$C_6H_{10}OH^+$	Hexenal/Methylpentenone/Hexanoic acid fragment(Aprea et al., 2015; Yener et al., 2016)	Aldehydes/Ketones/Acid fragment	1.14 ± 0.39
101.0601***	101.0603	$C_5H_8O_2H^+$	Pentenoic acid(Patiny & Borel, 2013)	Acids	2.60 ± 1.12
103.0401*	103.0396	$C_4H_6O_3H^+$	Acetyl acetate(Patiny & Borel, 2013)	Esters	0.14 ± 0.02
103.0755***	103.075	$C_5H_{10}O_2H^+$	Methylbutanoic acid/Pentanoic acid(Aprea et al., 2015; Yener et al., 2016)	Acids	1.05 ± 0.39
105.0357*	105.037	$C_4H_8OSH^+$	Methional(Yener et al., 2016)	Sulphur compounds	0.21 ± 0.06
105.0708***	105.070	$C_8H_8H^+$	Styrene/Ethylbenzene/Vinylbenzene(Yener et al., 2016)	Aromatic hydrocarbons	1.50 ± 0.71
109.066**	109.065	$C_7H_8OH^+$	Benzyl alcohol (cresol)(Yener et al., 2016)	Phenols	0.17 ± 0.05
109.1011***	109.101	$C_8H_{12}H^+$	Cyclooctadiene(Yener et al., 2016)	Hydrocarbons	2.01 ± 0.33
110.1045***	111.044	$C_6H_6O_2H^+$	Acetyl furan(Yener et al., 2016)	Furans	0.19 ± 0.03
117.0911***	117.0917/ 117.0913	$C_6H_{12}O_2H^+$	Ethyl butanoic acid/Hexanoic acid(Aprea et al., 2015; Bottiroli et al., 2020)	Esters/Acids	5.23 ± 1.98
123.0461**	123.0811	$C_8H_{10}OH^+$	Phenylethyl Alcohol	Alcohols	0.44 ± 0.13
125.0947***	125.0954	$C_6H_{10}N_3H^+$	2-Methoxyphenol (Guaiacol)(Patiny & Borel, 2013)	Phenols	0.20 ± 0.03
127.1119***	127.1488	$C_9H_{18}H^+$	2,4-Dimethylhept-1-ene	Alkenes	1.25 ± 0.08
136.1061***	131.107	$C_7H_{14}O_2H^+$	Heptanoic acid/Hexyl formate(Yener et al., 2016)	Acids/Esters	0.16 ± 0.05
137.1331***	137.133	$C_{10}H_{16}H^+$	Various monoterpenes(Yener et al., 2016)	Terpenes	1.10 ± 0.08
143.144*	143.143	$C_9H_{18}OH^+$	Nonanone/Nonanal(Aprea et al., 2015; Bottiroli et al., 2020; Yener et al., 2016)	Ketones/Aldehydes	0.79 ± 0.17
145.1232***	145.1230	$C_8H_{16}O_2H^+$	Ethyl hexanoic acid/Octanoic acid	Esters/Acids	2.27 ± 1.57
153.127***	153.127	$C_{10}H_{16}OH^+$	Decadienal(Yener et al., 2016)	Aldehydes	0.22 ± 0.02
165.091***	165.0917	$C_{10}H_{12}O_2H^+$	Acetic acid, 2-phenylethyl ester	Esters	0.04 ± 0.00

166.0876*	166.0869	$C_9H_{11}NO_2H^+$	Phenylalanine(Patiny & Borel, 2013)	Amino Acids	0.01 ± 0.00
173.1521***	173.1541	$C_{10}H_{20}O_2H^+$	Ethyl octanoic acid/n-decanoic acid(Aprea et al., 2015)	Esters/Acids	0.51 ± 0.34
201.1764***	201.1856	$C_{12}H_{24}O_2H^+$	Decanoic acid, ethyl ester	Esters	0.08 ± 0.03
IIIa001					
109.1011***	109.101	$C_8H_{12}H^+$	Cyclooctadiene(Yener et al., 2016)	Hydrocarbons	2.05 ± 0.12
110.1045***	111.044	$C_6H_6O_2H^+$	Acetyl furan(Yener et al., 2016)	Furans	0.19 ± 0.01
127.1119***	127.1488	$C_9H_{18}H^+$	2,4-Dimethylhept-1-ene	Alkenes	1.12 ± 0.05
IIIa004					
60.0216*	60.0212	$C_2H_3O_2H^+$	Acetate(Patiny & Borel, 2013)	Esters	0.73 ± 0.17
81.0697**	81.070	$C_6H_8H^+$	Cyclohexadiene(Yener et al., 2016)	Terpene fragment	1.49 ± 0.06
99.0806***	99.080	$C_6H_{10}OH^+$	Hexenal/methylpentenone(Yener et al., 2016)	Aldehydes	1.10 ± 0.11
109.1011***	109.101	$C_8H_{12}H^+$	Cyclooctadiene(Yener et al., 2016)	Hydrocarbons	2.05 ± 0.33
117.0911***	117.0917/ 117.0913	$C_6H_{12}O_2H^+$	Ethyl butanoic acid/ Hexanoic acid(Bottiroli et al., 2020)	Esters/Acids	5.09 ± 0.49
123.0461**	123.0811	$C_8H_{10}OH^+$	Phenylethyl Alcohol	Alcohols	0.43 ± 0.04
127.1119***	127.1488	$C_9H_{18}H^+$	2,4-Dimethylhept-1-ene	Alkenes	1.23 ± 0.12
136.1061***	131.107	$C_7H_{14}O_2H^+$	Heptanoic acid/Hexyl formate(Yener et al., 2016)	Acids/Esters	0.16 ± 0.02
153.127**	153.127	$C_{10}H_{16}OH^+$	Decadienal(Yener et al., 2016)	Aldehydes	0.19 ± 0.00
Tonga1					
69.0699**	69.070	$C_5H_8H^+$	Isoprene(Bottiroli et al., 2020; Yener et al., 2016)	Terpene fragment	23.85 ± 24.75
85.1008*	85.1018	$C_6H_{12}H^+$	Cyclohexane(Patiny & Borel, 2013)	Alkanes	0.49 ± 0.19
87.0801**	87.0811	$C_5H_{10}OH^+$	3-methyl butanal/2-Pentanone/Pentanal(Bottiroli et al., 2020)	Aldehydes/Ketones/Aldehydes	5.05 ± 5.45
93.0695**	93.070	$C_7H_8H^+$	Toluene(Bottiroli et al., 2020; Yener et al., 2016)	Aromatic hydrocarbons	1.06 ± 0.09
95.0855***	95.086	$C_7H_{10}H^+$	Methylcyclohexadiene (α-terpinene fragment)(Yener et al., 2016)	Terpenes	1.22 ± 0.61

103.0755*	103.075	$C_5H_{10}O_2H^+$	Methylbutanoic acid/Pentanoic acid(Aprea et al., 2015; Yener et al., 2016)	Acids	0.88 ± 0.26
111.0796***	11.080	$C_7H_{10}OH^+$	Heptadienal(Yener et al., 2016)	Aldehydes	0.37 ± 0.07
113.0962***	113.096	$C_7H_{12}OH^+$	Heptenal(Yener et al., 2016)	Aldehydes	3.02 ± 0.03
121.0656**	121.065	$C_8H_8OH^+$	Methylbenzaldehydecoumaran(Yener et al., 2016)	Aldehydes	0.85 ± 0.44
131.1067***	131.107	$C_7H_{14}O_2H^+$	Heptanoic acid/Hexyl formate(Yener et al., 2016)	Acids/Esters	2.77 ± 1.24
Tonga2					
93.0695**	93.070	$C_7H_8H^+$	Toluene(Bottiroli et al., 2020; Yener et al., 2016)	Aromatic hydrocarbons	1.04 ± 0.07
95.0855**	95.086	$C_7H_{10}H^+$	Methylcyclohexadiene (α-terpinene fragment)(Yener et al., 2016)	Terpenes	0.96 ± 0.13
111.0796**	11.080	$C_7H_{10}OH^+$	Heptadienal	Aldehydes	0.27 ± 0.05
113.0962**	113.096	$C_7H_{12}OH^+$	Heptenal(Yener et al., 2016)	Aldehydes	2.07 ± 0.39
131.1067***	131.107	$C_7H_{14}O_2H^+$	Heptanoic acid/Hexyl formate(Yener et al., 2016)	Acids/Esters	2.15 ± 0.39

*Significance code: *p-value ≤ 0.05 **p-value ≤ 0.01 **p-value ≤ 0.001*

Of the 390 mass peaks obtained, 218 were distinct for barotse4, and 35 mass peaks were distinct for barotse1. Backslopping1 and 4 had 31 and 9 mass peaks respectively, while only 5 mass peaks were distinct for illa1. Illa4 had 67 mass peaks. Tonga1 and tonga2 were described by 36 and 16 mass peaks, respectively. More of the tentatively identified compounds, including those with mass peaks at m/z 123.0461, m/z 165.0910, m/z 145.1232, m/z 117.0911, and m/z 201.1764, and identified tentatively as phenylethyl alcohol, acetic acid 2-phenylethyl ester, and decanoic acid ethyl ester significantly distinguished barotse4 from the other mabisi products in the PTR-QiTOF-MS analysis.

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CHAPTER

The effect of storage temperature on the functional characteristics and microbial community of traditionally fermented dairy products

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Submitted to Food Research International Journal

Abstract

Traditional fermentation is a widespread household practice among women and small-scale processors in rural communities. However, proper storage remains a challenge due to limited access to refrigeration, consequently, the products are often stored under ambient conditions before they are consumed. We assessed the effect of temperature on the quality characteristics and microbial community composition of two variants of a spontaneously fermented dairy product from Zambia known as *mabisi*. Samples were analyzed for their physicochemical components, including pH, total titratable acidity (TTA), and volatile organic compounds over a storage period of 24 days at refrigeration temperatures (chilled, 4.2°C) and 16 days at ambient temperatures (un-chilled, 25.8°C). The microbial counts and community composition were determined by culture-based and non-culture-based techniques. Bacterial communities were identified and classified by sequencing the amplicons of the V3–V4 region of 16S rRNA and yeast communities by internal transcribed spacer (ITS). Minimal variations in pH and TTA were found at the onset of storage. As post-acidification became more prominent towards the end of the un-chilled storage, a clear distinction was noticed between the chilled and un-chilled samples among the products regarding their volatile organic compounds, especially towards the end of storage. The bacterial communities for the two variants were stable at ambient and refrigeration temperatures, but the yeast communities varied markedly towards the end of the un-chilled storage with a drop in the total counts. Overall, limited post-acidification during chilled storage indicated the ability of low temperatures to enhance shelf life.

1.0 Introduction

Milk plays a vital role in human nutrition and is a source of revenue for pastoral households in many countries worldwide. However, the highly perishable nature of milk coupled with limited cold chain facilities and high temperatures that particularly occur in tropical climates, pose a challenge to its shelf life (Knight-Jones et al., 2016; Omore et al., 2001; Phiri et al., 2021). Spontaneous fermentation has been traditionally used as a low-cost method to extend the shelf life of milk while enhancing its quality and safety. Central to the production of traditional fermented milk products is the complex microbial community that drives the fermentation process. Comprising a diverse array of bacteria, fungi, and enzymes, these microbial communities play a pivotal role in converting raw ingredients into fermented food products, while imparting distinctive organoleptic characteristics (Holzapfel, 2002).

The fate of the microbial communities within fermented foods is essential to the product's quality, safety, and shelf life (Giraffa, 2004). Throughout the storage life of fermented foods, variations in storage conditions exert selective pressure on the communities of the fermenting microbes. This leads to changes in microbial community composition and may compromise the desired functional properties of the food. The microbes encounter a diverse range of selective pressures characterized by biotic and abiotic factors following the different processing methods and storage conditions (Mudoor Sooresh et al., 2023; Rolfe & Daryaei, 2020; Wolfe & Dutton, 2015). For instance, temperature fluctuations during storage can influence the dynamics of microbial communities, and govern biochemical and physical processes, and thus the overall characteristics of the product. Most milk-based fermented products are dominated by species of the genus Lactococcus sp. and Lactobacillus sp., which are typically mesophilic and thermophilic and experience reduced growth rates at chilled temperatures compared to ambient conditions (Delgado et al., 2013). Cold storage usually slows down metabolic and enzymatic activities and hinders microbial growth while elevated temperatures can support

these activities (Russell, 2002). Cold environments, such as freezing or chilled temperatures, are considered effective for preserving food products and maintaining their quality (Sun et al., 2020). This emphasizes the significance of temperature control for maintaining the quality and safety of fermented foods.

However, proper storage of food products remains a concern in rural Africa. Storage of several traditional fermented milk-based products such as amasi, nunu, mursik, and mabisi usually still occurs under ambient conditions as per their ancient practice and because of the limited cold storage facilities (Omore et al., 2001). In most cases, the quality of the products is maintained for up to several days without refrigeration. Yet, given the recent push for upscaling based on the perceived nutritional and health benefits associated with traditional fermented foods (Achi, 2005; Waché et al., 2021), there is an increasing interest in better control of product characteristics over longer periods. This is essential for scaling up production and facilitating increased sales through formal channels, thereby meeting the growing demand (Materia et al., 2021). Cold storage could extend these products' shelf life further, and benefit local producers by facilitating the sale of traditional fermented products beyond their communities or local markets.

In this study, we aim to understand the influence of temperature on the shelf life of mabisi and how product functionality is affected. Mabisi is an archetypical example of a milk-based traditional fermented food. Produced at the household level predominantly by local women, mabisi plays a crucial role in improving the food security and livelihoods of the local people by providing nutrition, revenue, and cultural value to local communities across Zambia. It is produced by the spontaneous fermentation of raw bovine milk at ambient temperatures. Production methods for several variants of mabisi and the different fermentation containers used have been outlined in previous studies (Moonga et al., 2019). Our study specifically investigated how the microbial community composition of the product changes as a function

of two distinct temperature regimes over a defined storage period. Refrigeration and ambient temperatures prevailing during the study period, which occurred in April and May, were selected to assess the microbial and quality attributes of two variants of mabisi: barotse and tonga mabisi. The ambient temperatures mirror the typical storage and transportation conditions for traditional mabisi observed among local processors and households. The products were evaluated over 16 days at ambient (i.e., un-chilled) and 24 days at refrigeration (i.e., chilled) temperatures. The study focused on analyzing the physicochemical components, particularly the pH, total titratable acidity (TTA), total viable counts (TVC), lactic acid bacterial (LAB) counts, and yeast counts. Amplicon sequencing of the V3 – V4 region of 16S rRNA and the internal transcribed spacer (ITS) were used to identify and classify the bacteria and yeast community structures in the stored samples. Based on the hypothesis that shifts in microbial composition could influence aroma profiles, the volatile organic compounds (VOC) were also monitored to give further insights into product functionality.

2.0 Materials and methods

2.1 Sample preparation

Two variants of mabisi, namely the barotse and tonga types, were prepared by following the traditional production methods (Moonga et al., 2019; Sikombe et al., 2023). In brief, tonga mabisi production involves the spontaneous fermentation of raw bovine milk at ambient temperatures for 24 - 48 hours. Production of the barotse variant involves an additional step of whey removal and the subsequent addition of fresh milk, typically repeated three to four times or until whey production is substantially reduced. Both mabisi variants were prepared and handled under similar conditions using raw milk collected from the field station of the School of Agriculture at the University of Zambia.

2.2 Experimental design

Following the production of *mabisi*, samples from the two product variants were stored for the specified duration under the two temperature variations as follows: 24 days under refrigeration (chilled) temperature (4.5 ºC) and 16 days under ambient (un-chilled) temperature (25.8ºC). The daily temperature fluctuation during the two storage conditions is presented in the supplementary material (Fig. S1). Samples were collected at intervals of four days with the initial sampling referred to as day 0. Overall, there were seven and five sampling time points for the chilled and un-chilled temperatures, respectively (Fig.1).

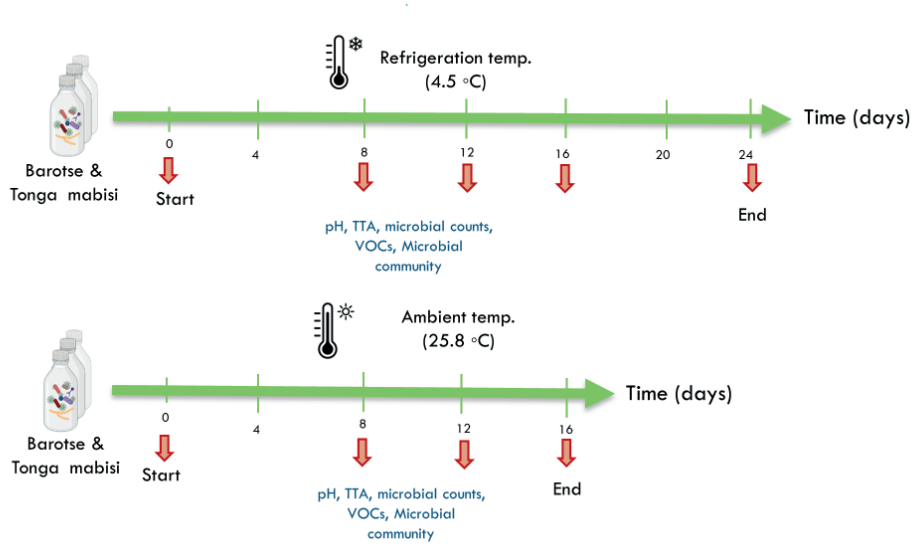


Fig. 1. The experimental design illustrating the storage of mabisi samples under two temperature regimes: refrigerated storage for 24 days and un-chilled storage for 16 days. These temperature variations reflect typical storage temperatures observed among households and local traditional *mabisi* processors. Samples collected on days 4 and 20 were not analyzed for VOCs and microbial communities.

2.3 pH and total titratable acidity (TTA)

The pH was measured by a digital pH meter (Hanna HI 8424) and total titratable acidity (TTA) according to the AOAC official methods (AOAC, 2005). The pH meter was calibrated using standard buffer solutions (Merck) at pH 4.0 and 7.0. TTA was calculated as a percentage of lactic acid after titrating 10 ml of mabisi against 0.1N standard NaOH to a faint pink color that stayed for 30 sec.

2.4 Volatile organic compounds

a) Data collection

Determination of the volatile organic compounds (VOCs) was by Headspace Solid Phase Microextraction Gas Chromatography-Mass Spectrometry (HS-SPME GC-MS) following the method described by Moonga and colleagues (2021). A TriPlus™ RSH autosampler coupled to a Trace™ 1300 GC and an ISQ™ Quadrupole MS (all three from Thermo Scientific™, Thermo Fisher Scientific Inc., Waltham, USA) was used for analysis. Frozen samples were equilibrated at 60°C for 20 min in the agitator of the autosampler. Volatile compounds were extracted for 20 min at 60°C using an SPME fibre coated with Carboxen, Divinylbenzene, and Polydimethylsiloxane (Car/DVB/PDMS) by Supelco™ (Thermo Fisher Scientific Inc., Waltham, USA). The compounds were desorbed from the fibre for 2 min onto a polar column (Stabilwax®-DA, 30 m length, 0.25 mm ID, 0.5 µm df, Restek, Bellefonte, USA). A Programmable Temperature Vaporizing (PTV) inlet was used as a sample inlet and heated to 250°C. It was operated in split mode at a ratio of 1:25. The GC oven temperature was kept at 35°C for 2 min, raised to 240°C with a slope of 10°C/min and kept at 240°C for 5 min. Helium was used as carrier gas at a constant flow rate of 1.2 mL/min. Mass spectral data was collected over a mass-to-charge ratio (m/z) range of 33–250 in full-scan mode with 3.0030 scans/sec.

b) Compound identification

Mass spectral data were analyzed using Chromoleon® 7.3.10 (Thermo Fisher Scientific Inc., Waltham, USA). The ICIS algorithm was used for peak integration with an area noise factor of 10, peak noise factor of 10, baseline window of 100, and Multiplet resolution of 3. The MS peak spectrum bunch setting was set to 3, and the peak dependent correction to 3, both left and right. The component table wizard was used to find components in the samples in a retention time frame of 2.5 to 22. The National Institute of Standards and Technology (NIST) main library from 2014 was used to match the components mass spectral profiles.

2.5 Microbiological analysis

a) Enumeration of viable microbial counts

Viable microbial counts were evaluated using three different microbiological culture media, namely plate count agar (PCA), de Man Rogosa Sharpe (MRS) agar, and potato dextrose agar (PDA) all from Merck, Germany. Samples were diluted in sterile Ringer´s solution. Serial dilutions were prepared, and 1 mL of the appropriate dilution was spread onto the relevant agar plates. Total viable counts (TVC) were determined after incubation on PCA at 37°C for 72 hours, Lactic Acid Bacteria (LAB) were incubated on MRS agar at 37°C for 72 hours, and yeasts were incubated on PDA supplemented with chloramphenicol at 30°C for 5 days. MRS plates were incubated in anaerobic jars. The colonies were counted after incubation and total counts were expressed as log cfu/mL.

b) DNA extraction

DNA was extracted from mabisi and purified following a previously described procedure (Schoustra et al., 2013). The quality and concentration of extracted DNA were checked and assessed using the NanoDrop™ ND-2000 and Qubit™ 4 fluorometer (Thermo Fisher Scientific, UK). The pre-checked DNA samples were dissolved in TE buffer and sent to Novogene (UK) for V3-V4 hypervariable region 16S rRNA and ITS amplicon sequencing on

the NovoSeq Illumina 6000 (strategy PE250) platform. Amplicons were produced using polymerase chain reaction (PCR) and the amplicon quality was checked by 2% gel agarose electrophoresis. PCR amplification of the target region was performed using the primers 341F CCTAYGGGRBGCASCAG and 806R GGACTACNNGGGTATCTAAT. Equal amounts of PCR products for each sample were pooled, end-repaired, A-tailed and ligated with Illumina adaptors. Paired-end reads were trimmed and filtered to obtain clean reads using fastp (version 0.20.0) software. Cleaned reads were generated using 250 base pairs (bp) paired-end raw reads using fast length adjustment of short reads (FLASH) software. Using the DADA2 open-source R package, sequences were paired and denoised. Chimeric sequences were removed using Vsearch (Version 2.15.0) software. The QIIME2 software (version QIIME2, 202006) was used to obtain the initial Amplicon Sequence Variants (ASVs). The microbial species annotation was performed with QIIME2 software using the Silva database as the annotation database. To examine the phylogenetic relationships between each ASV and the variations in dominant species across samples, QIIME2 software was used to align multiple sequences. ASV absolute reads were rarefied using a standard read number corresponding with the least reads. The ASVs abundance table was obtained at the level of the kingdom, phylum, class, order, family, and genus.

2.6 Statistical analysis

Physicochemical and microbiological counts results were expressed as the average of triplicate experiments and recorded as mean ± SD. Analyses were performed in Excel and R (Version 4.3.1). Significant differences between the results were calculated by analysis of variance (ANOVA) followed by a post-hoc Tukey test. Differences at $p < 0.05$ were considered to be significant. The VOCs data from the HS-SPME GC-MS were median normalized per compound and the heatmap function in the stats package (version 4.3.1) was used to visualize the data. The Phyloseq R package (version 1.46) was used for the microbiome data analysis

and the ggplot package (version 3.4.4) for generating graphs. The microbiome package (version 1.24.0) was used for calculating alpha-diversity, while the violin plots were visualized by ggpubr package (version 0.6.0).

3.0 Results

3.1 pH and total titratable acidity

The results of the pH and total titratable acidity (TTA) measurements of the mabisi samples during storage at chilled and un-chilled conditions are shown in Fig. 1(a) and (b). The pH values for the two mabisi variants did not vary much during storage ($p = 0.3905$). However, the chilled and un-chilled samples within each variant exhibited some notable variations towards the end of storage, particularly on days 16 and 20. The initial pH values for the barotse and tonga variants were 4.11 and 4.18, respectively. The pH in all the samples increased slightly from the initial values to pH 4.3 and 4.4 on day 4. Thereafter a drop was observed for the un-chilled samples. This decline continued steadily until days 16 and 20. The chilled samples, on the other hand, exhibited a pH decline until day 16, and an increase later between day 20 and 24. The final pH for the chilled samples was 4.4 on day 24 while that of the un-chilled samples was 3.78 for barotse and 3.53 for tonga. Product post-acidification was much higher in the un-chilled than the chilled samples.

Despite the absence of significant differences in the pH shifts between the two variants of *mabisi* at either of the temperatures during storage ($p = 0.3905$), the pH of the un-chilled tonga samples differed significantly between days 0 and 24 ($p < 0.05$). The chilled samples for both products exhibited higher and more stable pH values through the course of storage with low evidence of post-acidification, while the un-chilled samples presented a steady decrease in pH until the end of storage. Evidence of post-acidification was visually observed towards the end of un-chilled storage with the samples exhibiting more syneresis (Fig. S2.)

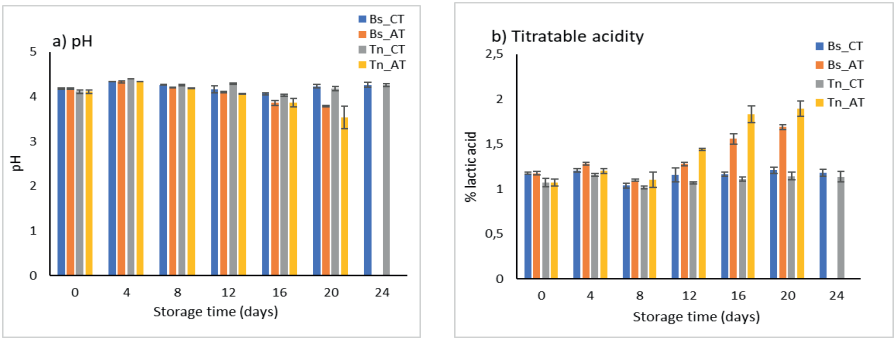


Fig. 2. Variations in the pH (a) and TTA (b) of the two types of *mabisi* during storage at refrigerated (CT) and ambient (AT) temperatures. Bs and Tn denote the barotse and tonga types, respectively.

The TTA results showed patterns that were in line with the pH results. TTA decreased as the pH increased over time. Whereas the TTA was stable up to day 8, differences became more vivid after day 12, especially for the tonga variant. The TTA increase was significantly higher in the un-chilled than the chilled samples, particularly on days 16 and 20 of storage. The chilled samples in both variants exhibited stable TTA values throughout most of the storage time. The TTA for barotse chilled samples was slightly higher than for the tonga chilled samples, while the reverse was true for the un-chilled samples.

3.2 Volatile organic compounds

A diverse range of volatile organic compounds was observed in the *mabisi* products and shifts during the period of storage were evident (Fig. 3a and b). The identified volatile compounds comprised 8 esters, 5 organic acids, 4 alcohols, and 5 carbonyl compounds. A distinction between the chilled and un-chilled samples was noticeable, especially for the barotse samples. The effect of temperature and storage time was more clear for the barotse than the tonga variant where some overlap was seen between the chilled and un-chilled samples. Un-chilled tonga samples from days 12 and 16 clustered together, while days 0 and 8 for both chilled and un-chilled tonga also formed a group and another cluster was formed by the chilled samples from

the remaining days. In un-chilled samples, an increase in the alcohol and esters compounds was observed towards the end of storage. For barotse, chilled samples clustered separately from the un-chilled samples. While the chilled barotse samples exhibited a steady increase in volatiles such as esters, organic acids, and aldehydes peaking on day 24 for most of the volatiles, the un-chilled samples showed an opposite pattern. In the un-chilled samples, most volatiles decreased over time, but there was an increased presence of higher alcohols and some esters. Moreover, the alcohol content tended to increase during un-chilled storage for both variants.

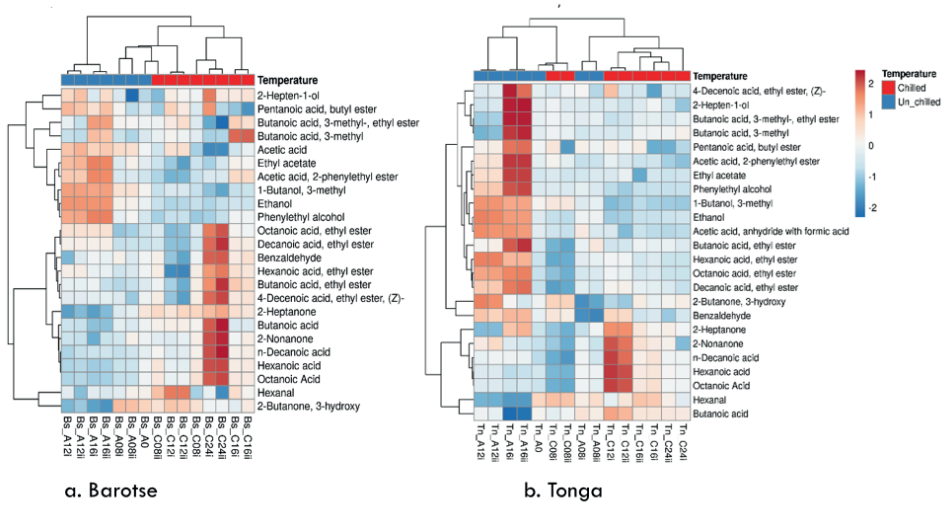


Fig.3. Heatmap of the correlation matrix of normalized volatile compounds data for the *mabisi* products during storage at refrigeration and ambient temperatures. Shifts in the volatile compounds of the chilled and un-chilled (a) barotse (b) tonga samples.

3.3 Viable microbial counts and community structure

a) Microbial counts during storage

Subtle differences were observed regarding the changes in the viable microbial counts from the beginning to the end of storage (Fig. 4). During the storage, the total plate counts ranged

between 7 and 9 log cfu/ml, reflecting a large variation in the size of the microbial communities. The communities included species from the genera *Lactococcus* sp., *Enterococcus* sp., *Lactobacillus* sp., *Weissella* sp., *Aeromonas* sp., *Streptococcus* sp., and several yeast species, as revealed by amplicon sequencing data. An initial decline in the viable counts was observed on day 4 followed by a gradual increase on day 8 and then a steady decrease towards the end of storage at both storage temperatures. The total viable count (TVC) values between temperatures showed no significant differences ($p > 0.05$). Between the two variants, tonga had higher TVC than the barotse variant.

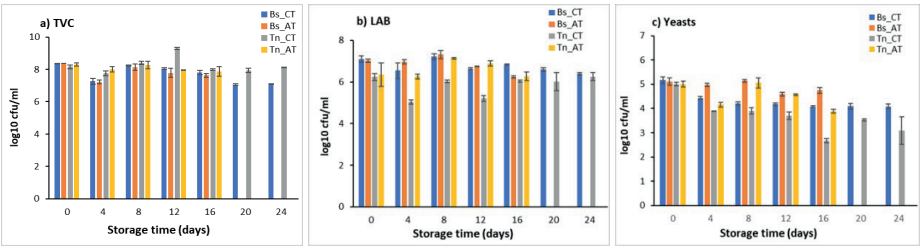


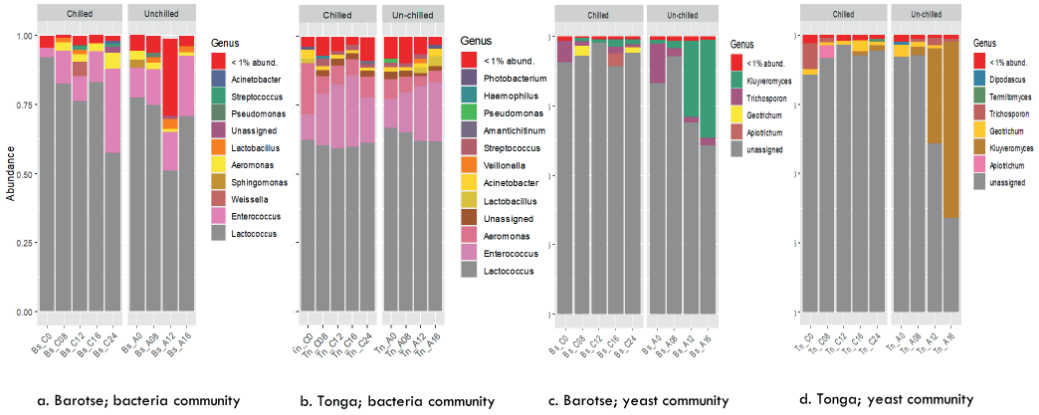
Fig. 4. Effect of storage temperature on the total viable bacterial counts (log cfu/ml). (a), Lactic Acid Bacteria (LAB) (log cfu/ml) (b), and yeasts (log cfu/ml) (c) in two types of *mabisi* products at AT: un-chilled temperature and CT: chilled temperature. Bs: Barotse; Tn: Tonga.

The LAB count for the barotse variant was mostly stable during the storage period. Between the temperatures, a significant drop was observed for the un-chilled samples on day 16 while the chilled samples remained stable. The chilled tonga samples maintained lower counts compared to the un-chilled samples with a significant temperature effect between day 4 and day 12 ($p < 0.05$). Although the microbial counts of the chilled tonga samples decreased somewhat during the middle of the storage, they recovered at the end. Day 8 recorded relatively higher counts compared to the other days for the two variants of *mabisi* regardless of the storage temperatures.

The viable yeast counts in all the samples varied between 2 and 5 log cfu/ml with an initial concentration for the two variants at around 5 log cfu/ml. A steady drop in the yeast count was generally observed from day 0 to day 24, although there was a significantly higher count for the un-chilled samples at day 8 ($p < 0.05$) for both variants. More specifically, the un-chilled barotse samples remained stable from day 0 to day 8 but steadily decreased between day 12 and day 16 while the chilled samples exhibited a steady decline up to day 8, followed by stability until the end of storage. Un-chilled tonga samples on the other hand showed fluctuating yeast counts between days 0 and 8, followed by a decrease between days 12 and 16. The chilled tonga samples showed a consistent decline throughout the storage period, with a notable drop observed on day 16.

b) Dynamics of the microbial community composition

Figure 5 illustrates the evolution of the bacterial community composition in the *mabisi* variants at the two different temperatures over time, as demonstrated by amplicon sequencing. Various LAB and other bacterial species were identified in the two mabisi variants. Both barotse and tonga were predominantly populated by members of the *Streptococcaceae* family, with the genus *Lactococcus* sp. being the most abundant. The genus *Enterococcus* sp. was the second most abundant group in all samples, followed by *Weissella* sp. in barotse and *Aeromonas* sp. in tonga, respectively. Other identified bacterial genera present in low abundances in the two variants included the former *Lactobacillus* sp., *Pseudomonas* sp., *Streptococcus* sp., and *Acinetobacter* sp. Additionally, *Sphingomonas* sp. was found exclusively in the barotse variant, while *Veillonella* sp., *Amantichitinum* sp., *Haemophilus* sp. and *Photobacterium* sp. were exclusive to tonga.



a. Barotse; bacteria community b. Tonga; bacteria community c. Barotse; yeast community d. Tonga; yeast community

Fig. 5. Relative abundances at the genus level during chilled and un-chilled storage at different time points for the bacterial communities of (a) barotse and (a) tonga samples, and the yeast communities of (c) barotse and (d) tonga samples.

In the barotse variant, the relative abundance of *Lactococcus* sp. decreased with time, irrespective of the storage temperature, while *Enterococcus* sp. increased with a higher relative abundance observed at the end of the storage. For the tonga variant, on the other hand, *Lactococcus* sp. was stable, maintaining a constant abundance over time, particularly in the chilled samples. Similar to barotse, *Enterococcus* sp. in the tonga variant increased steadily with time. The microbial community evolution in barotse was not as consistent as in the tonga variant where both chilled and un-chilled samples showed a similar trend of increasing abundance for *Enterococcus* sp.,while *Lactococcus* sp. mostly remained stable. *Aeromonas* sp. is seen as a potential spoilage organism and its presence is regarded as a safety hazard. *Aeromonas* sp. was most prominently present in the tonga variant and remained relatively stable during the storage period at both temperatures, and only appeared to diminish at the end of the un-chilled storage in the barotse variant.

The dynamics of the yeast communities during storage exhibited a different pattern than the bacterial communities. The yeast family *Dipodascacea* dominated the products throughout

storage. At the genus level, the most dominant species present in the two variants were unassigned. Most of these unassigned species, however, were from the family *Dipodascaceae*. The genus *Geotrichum* sp. was mostly observed in the chilled samples and less in the un-chilled samples, and showed a diminishing presence at the end of the un-chilled storage. However, towards the end of the un-chilled storage (days 12 and 16), the genus *Kluyveromyces* sp. started to dominate the un-chilled samples. *Trichosporonaceae* was the second most dominant yeast family and was represented by the genera *Apiotrichum* sp. and *Trichosporons* sp. While *Apiotrichum* sp. was mostly observed in the chilled samples, namely on day 16 for barotse and day 8 for tonga, *Trichosporons* sp. was mostly found in barotse day 0 samples. Their presence, however, diminished towards the end of the storage period for both variants.

The alpha diversity measure according to Simpson's index did not show significant differences in the microbial diversity and richness of samples at the two storage temperatures within each variant (Appendix Fig. S3). Although the difference observed was not significant, the chilled samples of both the barotse and tonga variants presented a highly diverse bacterial community compared to the un-chilled samples ($p > 0.05$). A similar pattern of diversity was observed for the yeast communities between the chilled and un-chilled samples (Fig. S4).

4.0 Discussion and conclusion

This study examined changes in the quality characteristics of a traditional fermented milk product during prolonged storage. Using *mabisi* from Zambia as an archetypical example, we focused on understanding how temperature influences the properties of traditional fermented milk products that are typically stored at ambient temperatures. Product quality characteristics such as the pH, titratable acidity, the metabolic output of volatile organic compounds, and the microbial population and community dynamics were followed for 16 days at ambient

temperature and 24 days at refrigeration temperature. The ambient temperature reflects the typical storage practices of households and local traditional mabisi processors.

The lowest pH value of 3.86 was recorded on the final day of un-chilled storage. In barotse samples, the TTA increased by 32.5%, and in tonga samples by 71.1%. The acidity of the products exhibited minimal variation at the onset of storage but as post-acidification became more pronounced towards the end of the un-chilled storage, the effect of the temperature difference became significant. The observed pH range corresponds to findings from previous studies on traditional mabisi and other African milk-based fermented products (Beukes et al., 2001; Moonga et al., 2021; Schoustra et al., 2013). This low pH range is desirable as it is considered to secure a safe product due to the ability to inhibit spoilage by the proliferation of undesirable microorganisms (Nout, 1994). Post-acidification suggests an active microbial metabolism and can lead to syneresis affecting product quality and shelf-life, and thus consumer appreciation (Deshwal et al., 2021). Conversely, the limited post-acidification during the chilled storage indicates the stability of the samples, highlighting the ability of low temperatures to enhance product resilience. Syneresis, though not quantified in this work, was observed toward the end of un-chilled storage, but only minimal signs were exhibited among the chilled samples (Fig. S2).

The size of the microbiological population increased during the early stage of the un-chilled storage period, followed by a decline towards the end of storage, as reflected in the TVC, LAB count, and yeast count. The declining microbial population could be a consequence of microbial death resulting from nutrient depletion. However, the influence of the difference in temperature at any given time point was generally minimal and not statistically significant, with only a few exceptions for the yeast populations. The marginal difference observed can be attributed to the robustness of the mabisi. Additionally, the similarities in microbial loads at the onset of storage may have contributed to the marginal differences observed over time. It is

worth noting that initial species abundance and diversity can affect the microbial communities' response to environmental fluctuations (Aubree et al., 2020).

In terms of microbial community composition, bacteria, particularly lactic acid bacteria (LAB), are the dominant components of the mabisi microbiome (Schoustra et al., 2013). This is evident from the higher microbial counts of LAB compared to the yeasts. Yeasts are a complementary group of microorganisms in traditional dairy fermentation (Quigley, O'Sullivan, et al., 2013). 16S rRNA sequencing confirmed that Streptococcaceae species, specifically from the genus Lactococcus, dominate the bacterial communities of both mabisi variants, irrespective of the storage temperature.

Among the tonga variants, the abundance of Lactococcus sp. remained relatively stable throughout most of the storage period, while a decline was more pronounced in the barotse samples towards the end of storage. The dominance of Lactococcus sp. can be attributed to the environmental conditions during the fermentation and storage of the products. Production and storage were between April and May, during which ambient temperatures in Lusaka, Zambia, fluctuated between 17 and 25°C (Appendix Fig. S1). Lactococcus sp. has shown adaptation to the prevailing ambient conditions in Africa (Jans et al., 2017). The declining Lactococcus sp. leading to a shift in abundance over time, particularly noticeable in the barotse variant, could be due to the presence of other competing microorganisms such as Enterococcus sp., and environmental pressures resulting in varied metabolic output (Ravyts et al., 2012).

Seasonal variations were not considered in this study, which may have influenced the outcomes. Different ambient temperatures could have altered the dynamics of the products, including, for example, causing excessive syneresis. Species of the genus Lactococcus sp. are homofermentative, mesophilic LAB, commonly found in various fermented dairy products, including cheese and many traditional milk-based African products (Beukes et al., 2001). These

species play a critical role in the fermentation and shaping of the aroma profile and other product characteristics of traditional mabisi, especially when production temperatures remain below 30°C (Moonga et al., 2021; Ravyts et al., 2012). The genus Enterococcus sp. as the next dominant species was present in appreciable abundance in both the barotse and tonga variants. While its abundance increased with storage time, the influence of temperature on the increase remained unclear, as apparent in both the chilled and un-chilled samples. This was more pronounced in barotse than tonga, where the increase in Enterococcus sp. was observed with the decline in Lactococcus sp. abundance. The increasing abundance of Enterococcus sp. with time shows its resilience and competitive advantage over other microbial species under storage conditions. Species of the genus Enterococcus sp. are also found in association with many other traditional fermented foods. Many authors view their presence as an indication of inadequate sanitary conditions during production and processing, often associated with foodborne illnesses. However, other researchers suggest that some species of the genus Enterococcus sp. are highly desirable in certain types of cheeses and other dairy products, as they positively contribute to the development of a unique flavor profile (Bhardwaj et al., 2009; Franz et al., 2011; Giorgio Giraffa, 2003; G. Giraffa, 2003; Goh & Philip, 2015).

The yeast community initially exhibited a degree of stability for both the barotse and tonga variants under the applied temperature variations. However, a distinct shift in the yeast dynamics marked by an increase in the genus Kluyveromyces was observed towards the end of un-chilled storage in both products. Given the high acidity recorded during this stage of storage, this could suggest the presence of acid-tolerant strains within this genus as there was an overall decrease in the yeast counts (Bilal et al., 2022). Despite their relatively small proportion within the mabisi microbial community, the shift in the yeast communities was accompanied by a moderate change in the metabolic profile.

An increase in specific volatile organic compounds was observed: alcohols (such as ethanol, phenyl ethyl alcohol, and 2-hepten-1-ol) and esters (such as ethyl acetate, butyl pentanoic acid, 3-methyl butanoic acid, and esters of hexanoic acid, butanoic acid, octanoic acid, and decanoic acid). This shift in the metabolic profile reflects the combined metabolic activity of the LAB and yeasts. Sensory evaluation during storage would provide further insights into how the shift and subsequent increase in volatile compounds influence the organoleptic quality of the products. While the chilled storage displayed greater diversity in both bacterial and yeast communities compared to the un-chilled storage, the difference between the two was not significant.

While a significant effect of the difference in storage temperature was reflected in pH and TTA values, the two variants of mabisi maintained their bacterial community composition during their storage life while the yeast communities decreased by more than 1 log. The bacterial counts and dynamics during storage did not significantly differ from the initial counts. This indicates that despite the shift in the functional properties, traditional mabisi remained relatively stable regarding the bacterial community during both the ambient and refrigeration storage. The stability and robustness of traditional mabisi are attributed to its complex microbial ecosystem dominated by LAB and complemented with yeasts. Nonetheless, refrigerated storage is recommended for preserving the desired qualities over a longer period. Moreover, assessing the consistency of the products would be essential, especially since syneresis was observed toward the end of the un-chilled storage period, which could significantly impact the textural properties of the products.

Our findings highlight the resilience of the natural and complex microbial communities present in traditional fermented products. Specifically, our study demonstrates that milk-based traditional fermented products exhibit a high degree of stability against differences in

temperature over a required storage period, emphasizing the importance of their diverse microbial communities in preserving product quality. This stability is attributed to the diverse microbial interactions among the different species within the community, which offer redundancy and resilience against such environmental fluctuations as temperature (Leale et al., 2023; Philippot et al., 2021). Functional redundancy within microbial communities allows different species to perform similar functions thus compensating for diminishing or lost species (Philippot et al., 2021). Additionally, the diverse nature of these communities enables them to cope with the varying environmental conditions they are exposed to.

Further investigations involving longer-term storage and storage under diverse environmental conditions, particularly taking into account seasonal variations, would yield additional insights into the intricacies of microbial population dynamics and their impact on product characteristics over time. This will not only enhance our understanding of traditional fermentation processes but also contribute to the development of strategies for optimizing product quality and stability in varying environmental contexts. In addition, it would provide a solid foundation for scaling up the production of traditional foods to contribute to food and nutrition security for numerous vulnerable households whose livelihoods depend on the traditional fermentation of food ingredients.

Acknowledgments

We would like to express our gratitude to the Integrated Research Fund (INREF) of Wageningen University and Research which funded the activities of this research. We would further like to thank the laboratory scientists and technicians from WUR and UNZA for the assistance assistance during the study.

Supplementary material

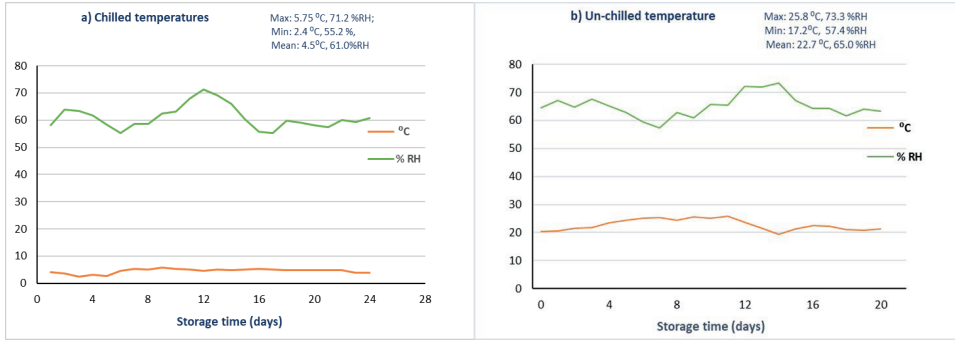


Fig.S1. Temperature variations during storage of mabisi based on the daily averages; (a) variations of the refrigeration (chilled) temperatures over a 24-day storage period and (b) variations of ambient (unchilled) temperatures during a 16-day un-chilled storage period.

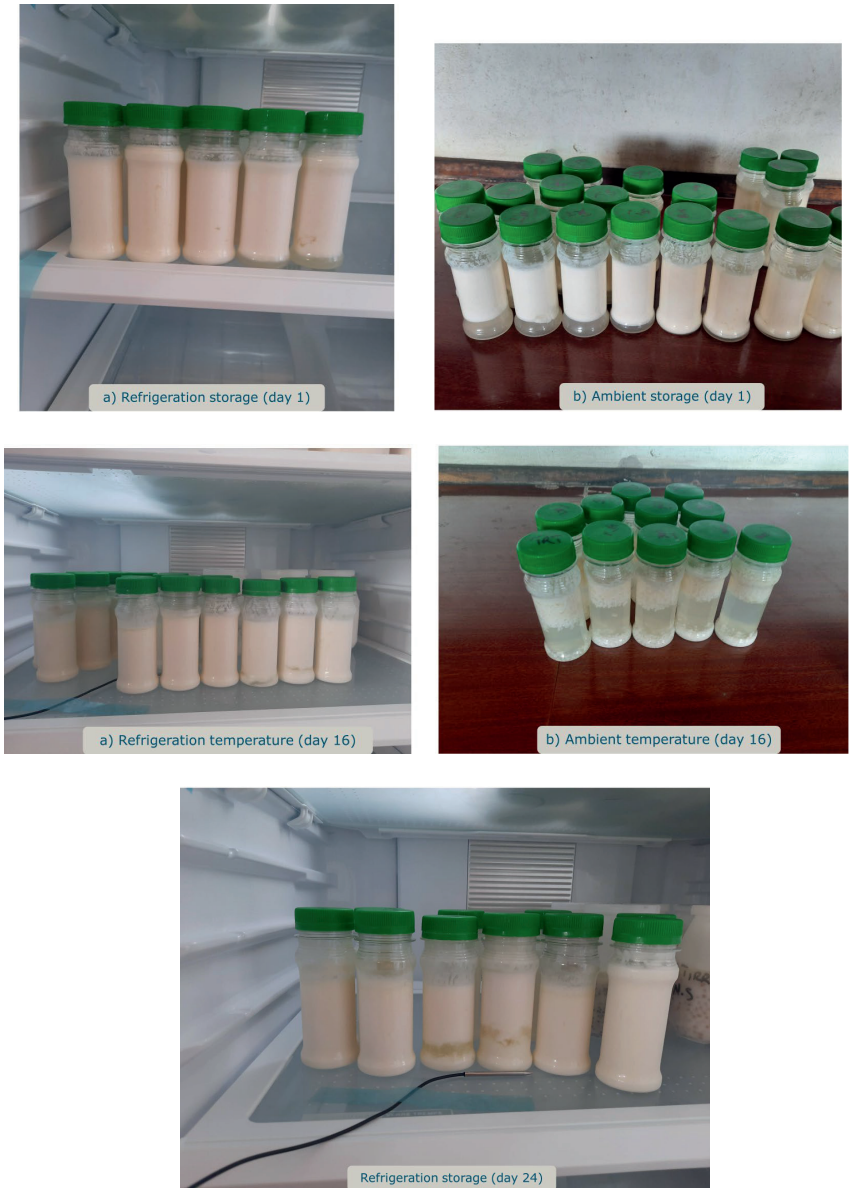


Fig. S2. Visual picture of the samples during the stages of storage showing product syneresis from day 1, day 14 (end of storage at ambient temperatures) and day 24 (end of storage for refrigeration temperatures)

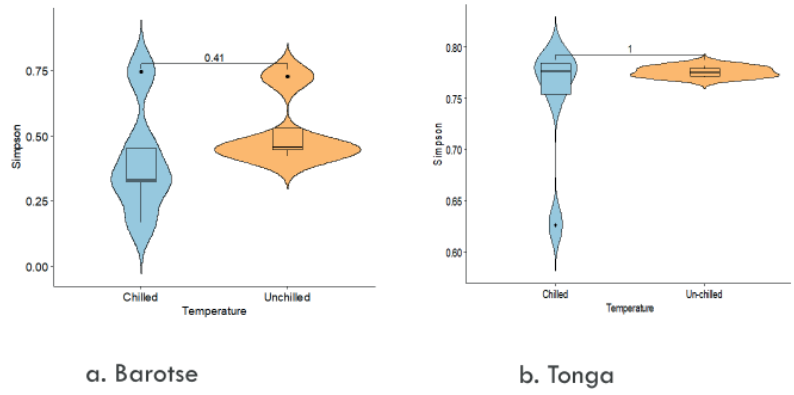


Fig.S3. Alpha diversities of the bacteria communities of the two mabisi variants at two storage temperature conditions according to the species richness (Observed) and Simpson diversity. The Simpson diversity shows that the chilled products were more diverse than the un-chilled products in both variants but not significantly different ($p > 0.01$).

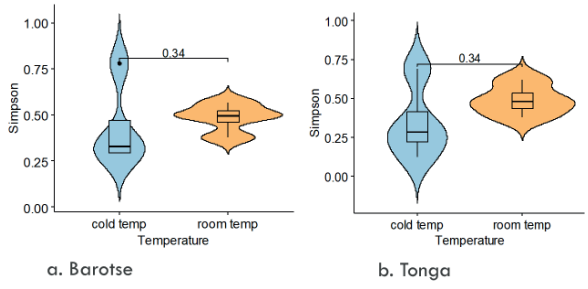


Fig.S4. Alpha diversities of the yeast communities of the two mabisi variants at two storage temperature conditions according to the species richness (Observed) and Simpson diversity. The Simpson diversity shows that the chilled products were more diverse than the un-chilled products in both variants but not significantly different ($p > 0.01$).

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CHAPTER

5

Isolation and evaluation of native mabisi microbial strains for starter culture development

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Abstract

We investigated the autochthonous microbial strains from mabisi, a traditional Zambian fermented dairy product with different variants. Mabisi is produced by the spontaneous fermentation of raw bovine milk at ambient temperatures for 24 to 48 hours. The spontaneous nature of mabisi production yields an end product with variable quality and stability. To determine the typical mabisi characteristics, we evaluated its autochthonous microbial strains. In total 67 microbial strains were isolated from two variants of mabisi and identified by sequencing the V3-V4 regions of the 16S rRNA gene for bacteria and the ITS region for the yeasts. The potential of these strains as starter cultures was assessed by their acidification capacity and volatile compound production in UHT milk.

Lactic acid bacteria (LAB) were the most dominant strains identified, along with a few yeast species. The LAB species included, in order of abundance, the genera *Enterococcus* sp., *Lactocaseibacillus* sp., *Lactiplantibacillus* sp., *Levilactibacillus* sp., and *Lactococcus* sp. The two identified yeast species were *Wickerhamiella* sp. and *Candida* sp. *Lactocaseibacillus* sp. exhibited the highest acidifying potential, followed by *Lactococcus* sp. Both *Lactococcus* and yeast contributed unique volatile organic compounds to the final product. In contrast, the contributions of *Lactiplantbacillus* sp. and *Levilactibacillus* sp. to the acidification of UHT milk were insignificant, although *Lactiplantbacillus* sp. showed some evidence of contributing to the volatile organic compounds.

1.0 Introduction

Lactic acid bacteria (LAB) are an important group of microbes in fermented food production (Teneva-Angelova et al., 2018). They are Gram-positive, non-sporulating organisms that belong to the phylum Bacillota. They are generally recognized as safe (GRAS) and are known to drive many traditional and industrial food fermentation processes (Franz et al., 2010). LAB form the natural microbiota in traditional fermented foods, each strain contributing unique functional and metabolic activities to the final product (Mozzi, 2016). The growing global interest in fermented foods has generated an increasing need to isolate new LAB strains for developing innovative products. The biodiversity and distinct characteristics of traditional fermented food microbiota make them a valuable reservoir of autochthonous cultures, suitable for application in various fermentation processes (Vera-Pingitore et al., 2016).

Mabisi is a traditional fermented product made by the spontaneous fermentation of raw bovine milk using the native microbes in milk, the utensils and equipment used for processing. It is typically produced at ambient temperature and sometimes backslopping with an aliquot from a previous successful batch is used to initiate the fermentation (Leroy & De Vuyst, 2004; Moonga et al., 2019). The quality of the raw milk, environmental conditions, and the producer influence the microbial community composition of mabisi (Moonga et al., 2020). During fermentation, interactions take place among autochthonous microorganisms, which are responsible for shaping the final product's characteristics and properties (Smid & Lacroix, 2013). The distinct characteristics of the final product are linked to different production techniques, deeply rooted in tradition, and influened by regional variations (Moonga et al., 2020). This is reflected by the varying consumer preferences for different types of mabisi across diverse demographics (Sikombe et al., 2023).

Spontaneous fermentations lack batch-to-batch consistency in product quality and stability, coupled with the potential for failure, which raises concerns for large-scale production. To mitigate such risks, strict control measures must be implemented, or alternatively, a careful selection of starter cultures for production could be considered (Leroy & De Vuyst, 2004). Characterizing the indigenous microbiota of traditional fermented foods (such as mabisi)_and developing tailored starter cultures are essential for optimizing and scaling up production. Moreover, the application of native starter cultures could help produce culturally adapted and innovative alternatives with desirable flavours and qualities to serve a larger market. Mabisi is an archetypical example of a traditional fermented food for which starter cultures have not been developed, hampering further optimization and upscaling.

During milk fermentation, lactose is converted into lactic acid by LAB species present in raw milk or introduced as starter cultures, thereby increasing the total acidity. Inoculating the substrate with starter cultures ensures a controlled and rapid onset of acidification. Rapid acidification inhibits the growth of undesirable and potentially harmful microorganisms by quickly lowering the pH of the substrate, creating a safer product with an extended shelf life (Nout, 1994; Smid & Kleerebezem, 2014). Additionally, controlled acidification contributes to the development of the desired texture and consistency in the final product, particularly in dairy products where curd formation and whey expulsion are essential processes (Abarquero et al., 2022). Furthermore, the use of starter cultures allows for control over the production of volatile organic compounds, which is crucial for defining the product's aroma profile (Hu et al., 2022). Thus, producers can consistently create products with desirable sensory attributes and other properties by selecting specific strains known for their ability to produce particular compounds.

Acidification and the metabolic formation of volatile compounds are crucial fermentation parameters that determine the quality and acceptability of dairy products. Identifying strains

with desirable characteristics in these aspects is essential for developing effective starter cultures. Since the metabolic functions of LAB are diverse, each strain plays multiple roles in dairy fermentation, resulting in variations in the metabolite profile. In this respect, LAB strains that efficiently produce acid and generate a pleasant aroma profile are highly valued. Other essential attributes considered in strain selection include vitamin biosynthesis, probiotic and antimicrobial properties, and polysaccharide production (De Angelis & Gobbetti, 2016; Surve et al., 2022).

This study explored the potential of using native microbial strains isolated from traditional mabisi in developing a starter culture to produce a fermented milk product with typical mabisi characteristics. The functionalities of the strains were evaluated based on their acidifying potential and ability to produce important volatile organic compounds in UHT milk. Although the natural microbial community of traditional dairy products is dominated largely by LAB, yeasts are also present in low abundance and are associated with unique flavour formation (Quigley, McCarthy, et al., 2013; Schoustra et al., 2013). In our study, we therefore complemented the starter cultures with one yeast species. The strains were tested individually in monoculture fermentations, as a mixed-strain culture comprising four bacterial strains and one yeast strain, and in mixed cultures with the omission of one strain at a time.

2.0 Methods

2.1 Preparation of mabisi samples

Two variants of traditional mabisi, prepared by the production methods outlined in previous studies, were used (Moonga et al., 2019; Sikombe et al., 2023). Briefly, raw bovine milk was spontaneously fermented at ambient temperatures for 24 - 48 hours for the so-called Tonga mabisi. The Barotse variant production involved an additional step of whey removal with the subsequent addition of fresh milk, typically repeated three to four times or until whey

production was substantially reduced. Both mabisi products were prepared and handled under similar conditions, using raw milk collected from the field station of the School of Agriculture at the University of Zambia.

2.2 Isolation of bacteria and yeast from traditional mabisi

One millilitre of mabisi sample mixed with 9 mL phosphate buffered solution (0.85% w/v, NaCl) was vortexed to make an initial dilution. Serial dilutions were made for each sample followed by plating on their appropriate media (MRS, M17, or PDA) to cultivate the LAB and yeasts. Cycloheximide at a concentration of 0.01% (v/v) was added to the MRS plates, and PDA was supplemented with chloramphenicol (0.1 g/L). MRS and M17 plates were incubated under anaerobic conditions at 30 ℃ for 48-72 hours and plates was incubated at 25 ℃ for 5 days in the case of PDA. Colonies with distinct morphological differences (based on colour, shape, size, and appearance of the surface (rough or smooth)) were randomly picked from the highest dilution and purified by successive streaking on the relevant media. The resulting isolates were stored at -80 ºC in MRS broth with 20% glycerol.

For the PCR amplification, each reaction contained DNA template of a single colony picked with a micropipette tip (≈ 1ul), 4 µl of 5X GoTaq Buffer, 0.5 ul Forward primer (10uM) 341F CCTAYGGGRBGCASCAG, 0.5 ul Reverse primer (10uM) 806R GGACTACNNGGGTATCTAAT, 0.8 ul dNTP 5mM 0.1 ul Taq DNA polymerase, and 13.1 ul H2O. DNA amplification was carried out in a PCR thermal cycler with the following conditions 95 ºC for 3 min, followed by 95 ºC for 30 sec, 58 ºC for 30 sec, 72 ºC for 1 min, followed by 29 cycles of 30 seconds at 95 ºC, 5 min at 72 ºC. For the yeast isolates, ITS1F CTTGGTCATTTAGAGGAAGTAA ITSR GCTGCGTTCTTCATCGATGC. DNA amplification was run with the following conditions: 95 ºC for 3 min, followed by 95 ºC for 30

sec, 55 ºC for 30 sec, 72 ºC for 1 min, followed by 34 cycles of 30 seconds at 95 ºC, 5 min at 72 ºC. Appropriate positive and negative controls were included in the test.

2.3 DNA sequencing

The amplicons of V3/V4 regions of the 16S rRNA gene of the bacteria isolates and the internal transcribed spacer (ITS) region of the yeast isolates were obtained by colony PCR. The PCR products were submitted to Eurofins Genomics (NL) for Sanger sequencing. Geneious Prime was used to align the sequences and to identify the strains using BLAST (Basic Local Alignment Search Tool) and the NCBI ('16S ribosomal RNA') database. Sequences with more than 98% similarity were considered to belong to the same species. However, the sequences were too short for taxonomic identification on a species level, and therefore could only be identified up to the genus level. The isolated strains of bacteria were plated on MRSA and incubated at 30°C (micro-anaerobe) while yeast was plated on Malt extract agar (MEA) at 25 °C. After 3 days of growth, the colonies were stored in cryovials containing 300 ul glycerol and 700 ul isolate broth and k at -20°C until required.

2.4 Preparation of strains for defined starter cultures

The bacterial strains from frozen stock were streaked onto an MRS agar plate using a sterile inoculation loop and incubated overnight (48 hours) at 30 °C. Pure isolated colonies were selected from the plate and grown overnight in 10 mL MR broth (Merck) at 37 °C with shaking at 160 rpm. The yeast colonies were cultured on malt extract agar and broth (Oxoid) at 28 °C in a shaker at 160 rmp. All broth tubes were incubated for 24 hours. The samples were prepared by inoculating 100 ml UHT full-fat milk with the pre-determined concentrations of strain cultures. The strain concentrations are displayed in the Table. 1. The preparations included monoculture and single-strain omission inoculation with milk and the inoculation with a complete starter culture comprising four bacterial and one yeast strains i.e *Lacticaseibacillus*,

Lactiplantibacillus, *Levilactibacillus,* and *Lactococcus*, and *candida* species. The fermentation was performed at 25 °C for 48 h.

Table 1. Recipes for the prepared culture fermented samples. Each sample was made by inoculating 100 ml UHT Milk with the appropriate concentrations of different strains as displayed. Complete starter: *Lacticaseibacillus, Lactococcus*, *Levilactobacillus*, *Lactiplantibacillus*, and *Candida* (yeast).

	Strain Id	**Strain concentration**					**Total concentration**
		Log 10 cfu/ml					
1	Lacticaseibacillus	8.11					8.11
2	Lactiplantibacillus		8.83				8.83
3	Lactococcus			8.60			8.60
4	Levilactobacillus				8.85		8.85
5	Yeast					7.25	7.25
6	No Yeast	7.64	8.23	8.00	8.25		8.69
7	No Levilactobacillus	7.73	8.32	8.09		6.79	8.60
8	No Lactococcus	7.73	8.32		8.34	6.79	8.69
9	No Lactiplantibacillus	7.73		8.09	8.34	6.79	8.61
10	No Lacticaseibacillus		8.32	8.09	8.34	6.79	8.75
11	Complete Starter	7.49	8.21	7.98	8.23	6.55	8.67

2.5 pH measurements

The pH was measured at 12-hour intervals using a digital pH meter (Hanna HI 8424). The pH meter was calibrated using standard buffer solutions (Merck) at pH 4.0 and 7.0.

2.6 Volatile organic compounds

a) Data acquisition

Determination of the volatile organic compounds (VOCs) was performed by using the Headspace Solid Phase Microextraction Gas Chromatography-Mass Spectrometry (HS-SPME GC-MS) following the method described by Moonga and colleagues (2021). A TriPlus™ RSH autosampler coupled to a Trace™ 1300 GC and an ISQ™ Quadrupole MS (all three from Thermo Scientific™, Thermo Fisher Scientific Inc., Waltham, USA) was used for analysis.

Frozen samples were equilibrated at 60 °C for 20 min in the agitator of the autosampler. Volatile compounds were extracted for 20 min at 60 °C using an SPME fibre coated with Carboxen, Divinylbenzene, and Polydimethylsiloxane (Car/DVB/PDMS) by Supelco™ (Thermo Fisher Scientific Inc., Waltham, USA). The compounds were desorbed from the fibre for 2 min onto a polar column (Stabilwax®-DA, 30 m length, 0.25 mm ID, 0.5 µm df, Restek, Bellefonte, USA). A Programmable Temperature Vaporizing (PTV) sample inlet was used, heated to 250 °C and operated in split mode at a ratio of 1:25. The GC oven temperature was kept at 35 °C for 2 min, raised to 240 °C with a slope of 10 °C/min and kept at 240 °C for 5 min. Helium was used as carrier gas at a constant flow rate of 1.2 mL/min. Mass spectral data was collected over a mass-to-charge ratio (m/z) range of 33–250 in full-scan mode with 3.0030 scans/second.

b) Compound identification

Mass spectral data were analyzed using Chromoleon® 7.3.10 (Thermo Fisher Scientific Inc., Waltham, USA). The ICIS algorithm was used for peak integration with an area noise factor of 10, peak noise factor of 10, baseline window of 100, and Multiplet resolution of 3. The MS peak spectrum bunch setting was set to 3, and the peak dependent correction to 3, both left and right. The component table wizard was used to find components in the samples in a retention time frame of 2.5 to 22. The National Institute of Standards and Technology (NIST) main library from 2014 was used to match the components´ mass spectral profiles with the NIST mass profiles.

2.6 Data Analysis

Microsoft Excel was used for data processing, and creating figures. Geneious Prime software was used to combine forward and reverse primer sequences and strain identification. Chromeleon (v.7) was used to identify aroma compounds from HS-SPME GC-MS. R Studio

(v. 4.3.1) the 'FactoMineR' package was used to create PCA (Principal Component Analysis) plots. The VOCs data were median normalized per compound and the heatmap function in the stats package was used to visualize the data.

3.0 Results

3.1 Identification of isolated strains

A total of 67 strains were isolated from two variants of traditional mabisi, consisting of 58 bacterial and 9 yeast strains. The isolates were identified at the genus level using the amplicon sequencing of 16S rRNA encoding DNA for the bacteria and internal transcribed spacer (ITS) for the yeasts. The identified isolates resulted in five LAB species and two yeast species. The isolates on MRS and M17 media were LAB species belonging to the genera *Enterococcus* (36%), *Lacticaseibacillus* (33%), *Lactiplantibacillus* (17%), *Levilactibacillus* (7%), and *Lactococcus* (5%). The identified yeasts were primarily *Wickerhamiella* (56%) and *Candida* (33%), with the remaining being unidentified. Yeasts were exclusively isolated from the Tonga variant of mabisi as there was no yeast growth in the Barotse samples. Figure 1 represents the genus distribution of the 67 isolated strains. Four bacterial strains from the genus *Lacticaseibacillus*, *Lactiplantibacillus*, *Levilactibacillus,* and *Lactococcus*, and one yeast strain were selected to constitute a complete mixed-strain starter culture for a series of fermentation experiments using UHT milk as a substrate to evaluate the performance of the starters based on their acidification capacity and production of volatile organic compounds (VOCs).

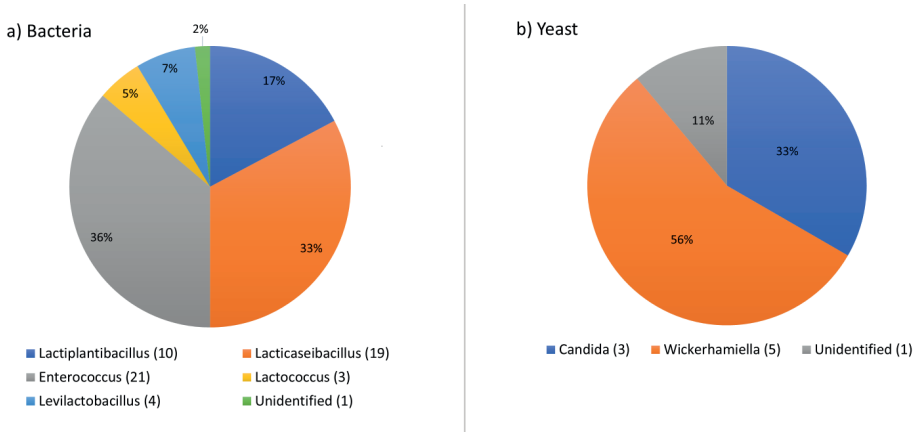

Fig. 1. The percentage (%) distribution of the 67 isolated microbial strains from mabisi identified at the genus level. The total isolate number per strain is indicated in parentheses after the strain name; (a) Bacteria strains and (b) Yeast strains.

3.2 Acidification capacity

A series of fermentations were conducted with monoculture, complete mixed-strain culture, and complete culture with single-strain omission using the isolated strains of *Lacticaseibacillus* sp., *Lactococcus* sp., *Levilactobacillus* sp. and *Lactiplantibacillus* sp., and one yeast strain (*Candida*). The strains were evaluated for their ability to effectively ferment UHT milk as mono-cultures and as part of a complete mixed-strain culture. Additionally, single-strain omissions from the five-mixed-strain culture were tested. Table 1 shows the concentrations of inoculum strains used. Figure 2 displays the pH values as an indicator of the acidification capacity of the strains. The four bacterial isolates exhibited diversity in their acidifying potential as monocultures in UHT milk, while the yeast strain showed minimal acidifying potential. The final pH of the fermented UHT milk, inoculated with monocultures of each of the individual strains, and incubated at 25 ºC for 48 hours, ranged from 4.33 to 5.71. Fermentations with mixed cultures, including single omission cultures, resulted in pH values that ranged from 4.34 to 4.98. The five-strain mixed culture had a final pH of 4.34 after 48 hours. As a monoculture, *Lacticaseibacillus* sp. had the lowest pH value of 4.33 after 48 hours

of incubation at 25 ºC, similar to the final pH of the complete mixed strain culture. When *Lacticaseibacillus* was left out of the complete starter, the final pH reached a value of 4.98 (Fig. 2b), highlighting the significant contribution of *Lacticaseibacillus* sp. in the acidification of milk. *Lactococcus* sp. as a monoculture reduced the pH to 5.39. Without *Lactococcus* sp. in the starter culture, the pH only dropped to 4.53. A final pH of 5.09 was observed during the monoculture fermentation of *Lactiplantibacillus* sp., and its omission from the complete starter culture did not yield any noticeable difference. *Levilactibacillus* sp. showed the least pH reduction to 5.71 after 48 hours, and its omission from the starter culture resulted in no noticeable difference. The largest differences from the single omission were observed upon omitting *Lacticaseibacillus* sp. and *Lactococcus* sp. from the starter culture. In contrast, with the omission of *Lactiplantibacillus* sp. or *Levilactobacillus* sp., a final pH of 4.34 was still achievable.

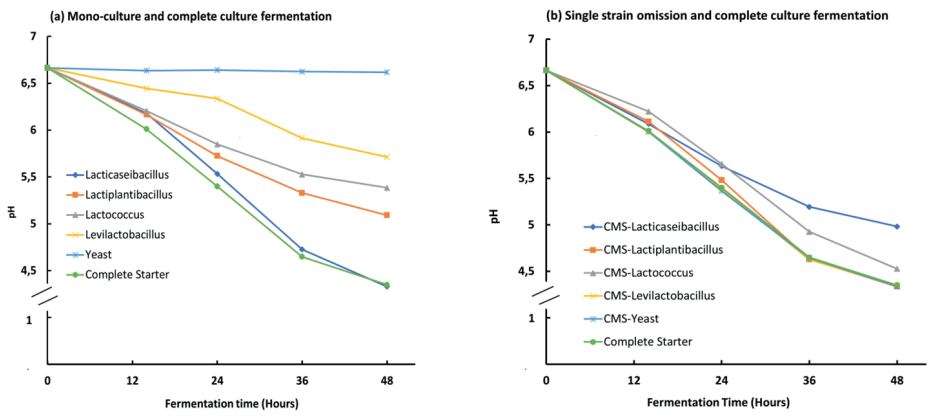

Fig. 2. Acidification of UHT milk during fermentation with strains isolated from traditional mabisi: (a) pH during monoculture and five-strain complete starter culture fermentation of *Lacticaseibacillus, Lactococcus*, *Levilactobacillus*, *Lactiplantibacillus*, and yeast (candida); (b) pH during fermentation with single strain omission and the complete starter. Incubation was for 48 hours at 25°C.

Given that the desired pH of 4.2 could not be achieved in the experiment, we tested an increased fermentation temperature of 28 ºC and an extended fermentation time of 72 hours at 25 ºC (Fig.

S1). The fermentation at 28 ºC proceeded faster than at 25 ºC with a final pH of 4.32 after 48 hours. Extended fermentation at 25 ºC had a pH of 4.09 after 72 hours compared to 4.48 after 48 hours. The two fermentation temperatures showed a very similar pH pattern with a difference of 0.2 units until 48 hours of fermentation.

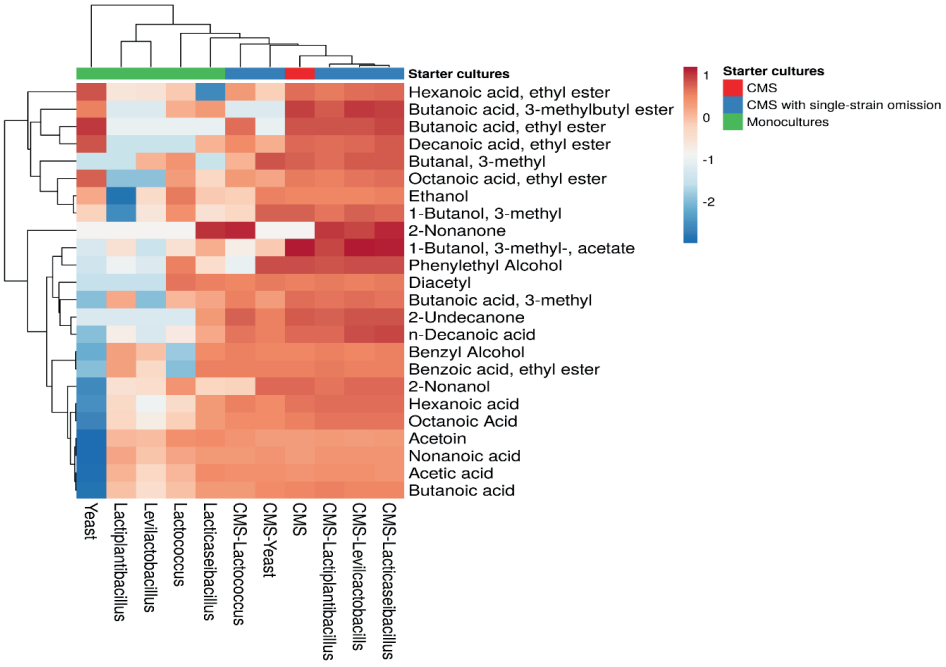

Fig. 3. Heatmap matrix showing the volatile profiles of the monoculture and single omission samples using the mabisi strains as starter cultures in UHT milk. The average peak area percentages of each compound per sample is given with the colour gradient: deep red represents the lowest and deep green represents the highest average normalized peak area percentages.

3.3 Volatile organic compound profiles of the isolates

The volatile organic compounds produced during monoculture, mixed-strain culture, and single-strain omission culture fermentation were determined using HS-SPME-GC/MS. Various volatile compounds were produced including esters, organic acids, carbonyl

compounds and alcohols. *Lactococcus* sp. and yeast exhibited a unique profile of volatile compounds with desirable attributes that are known to impart the characteristic odour-active mabisi aromas (Chapter 3 of this thesis). Figure 3 presents a heatmap depicting the similarities and differences among the volatile organic compounds. A monoculture of yeast exhibited a distinct volatile compound profile, characterized by several esters such as butanoic acid ethyl ester, hexanoic acid ethyl ester, octanoic acid ethyl ester, and decanoic acid ethyl ester. These compounds were either absent or present in low quantities during the fermentation of other monocultures and were only detected in such substantial amounts in the fermentation with a complete mixed-strain culture or the single-strain omission of *Lacticaseibacillus* sp., *Lactiplantibacillus* sp., and *Levilactobacillus* sp. Similarly, the *Lactococcus* sp. monoculture yielded unique compounds such as ethanol, diacetyl, acetoin, 1-butanol 3-methyl, 2-nonanol, and phenylethyl alcohol that were only detected in the complete mixed-strain culture, and in insignificant amounts when *Lactococcus* sp. was omitted from the starter culture. On the other hand, *Lacticaseibacillus* sp. seemed to be partially involved in the production of diacetyl and acetoin, but was mostly associated with acetic acid, hexanoic acid, octanoic acid, and nonanoic acid. It was also associated with the absence of hexanoic acid ethyl ester and butanal, 3-methyl. While *Lactiplantibacillus* sp. was associated with acetic acid, nonanoic, and benzyl alcohol, *Levilactobacillus* sp. did not appear to contribute significantly to the volatile profile of the complete starter culture, as it exhibited no unique patterns in a monoculture, and a similar profile to the complete mixed-strain culture was maintained even during its absence.

4.0 Discussion

To assess the feasibility of designing a starter culture using autochthonous strains from traditional mabisi, we managed to isolate a series of strains from traditional mabisi. The strains we isolated are common species previously found in various natural environments such as plants, meat, and fermented dairy products (Akabanda et al., 2014; Beukes et al., 2001; Gadaga

et al., 2000; Mathara et al., 2004; Sawadogo-Lingani et al., 2010). Only five species of LAB and two species of yeast were identified, which is considered a relatively lower number compared to what has been found before in other studies (Akabanda et al., 2010; Sawadogo-Lingani et al., 2010). The LAB strains identified are all homofermentative species that produce lactic acid as the main end product of fermentation (Wang et al., 2021). Various functional properties, including exopolysaccharide production, antimicrobial activity, vitamin biosynthesis, and probiotic potential are attributed to these strains (Surve et al., 2022; Tarannum et al., 2023). Previous studies investigating the microbial community of mabisi have revealed a diverse community of microbes responsible for fermentation. Using culture-independent methods, the studies reported mabisi to be largely dominated by members of the genus *Lactococcus* sp. and the formerly Lactobacillus sp., while *Enterococcus* sp., which was the most dominant species isolated in our study, was typically present in lower abundance (Moonga et al., 2019; Schoustra et al., 2013). Similar traditional dairy products of African origin have also recorded **Lactococcus** sp. and *Lactobacillus* sp. among the dominant species (Beukes et al., 2001). Acetic acid bacteria have also been reported in somewhat low abundance (Moonga et al., 2020), but we could not isolate any in our study.

In addition to bacteria, yeasts also play a role in traditional fermentation processes. They have been identified as low-abundance species in mabisi and other traditional fermented dairy products (Hebert et al., 2000; Quigley, O'Sullivan, et al., 2013; Schoustra et al., 2013). The previous studies on mabisi also indicated that the microbial community in spontaneously fermented mabisi varied depending on the geographical location and the production techniques used by the producer (Moonga et al., 2020). The discrepancy in observing a higher number of *Enterococcus* sp. compared to the other species may be due to bacterial pleomorphism, as some strains might have been isolated multiple times since strain selection from the agar plates was based only on morphological appearance. Moreover, among the five identified genera,

Lacticaseibacillus sp., *Lactiplantibacillus* sp., and *Levilactobacillus* sp. were previously classified under the genus *Lactobacillus* (Zheng et al., 2020). Despite *Enterococcus* sp. being frequently reported in mabisi and other traditional fermented dairy products, it is known to harbour some virulent traits (Franz et al., 2003). Due to these safety concerns, *Enterococcus* sp. was excluded in our study from the strains used to form the mixed strain starter culture.

Acidification is important in shaping the microbial landscape and developing desirable characteristics in the final food product (Parente et al., 2017). The fermentation of monocultures and single-strain omission cultures was particularly useful in assessing the individual contributions of each strain and their role within the mixed strain culture. The variations in acidifying capacities among different strains highlight their distinct contributions to fermentation and their potential impact on the quality of the final product, both as monocultures and within mixed cultures. Notably, *Lactococcus* sp., despite its strong acidifying potential, did not perform well as a monoculture. However, its omission from the mixed strain culture resulted in a higher final pH, underscoring its relevance to overall pH reduction. Mixed strain cultures exhibit various microbial interactions mediated through a series of molecular and physiological mechanisms (Ravyts et al., 2012; Smid & Lacroix, 2013). These interactions are crucial for modulating the characteristic properties of the final product. Consequently, the performance of individual strains in a monoculture appears to differ significantly from their performance in a consortium with other microbes. The behaviour of Lactococcus sp. may exemplify these interactions, affecting its metabolic behaviour and acidifying potential. Additionally, our experiment might have used a strain of *Lactococcus* sp. with low acidification capacity, as rapid acidification is typically a characteristic of L. lactis (Li et al., 2020; van de Bunt et al., 2014). Lacticaseibacillus sp., known for its strong acidifying potential, exhibited good performance compared to *Lactiplantibacillus* sp. and *Levilactibacillus* sp. Its omission from the complete starter culture also resulted in a higher

final pH. In contrast, omitting *Lactiplantibacillus* sp. and *Levilactibacillus* sp. showed no significant difference in the acidification patterns, as these strains had weak acidifying potential. The contribution of yeast to the acidification of milk was negligible, suggesting the importance of LAB as facilitators of fermentation and the ultimate chemical composition. Although the desired pH of 4.2 was not reached after 48 hours, extending the fermentation time to 72 hours, which is sometimes typically done for Tonga mabisi, successfully achieved the target pH.

The mixed-strain starter culture had far more abundant volatile compounds than the monocultures. However, a more abundant volatile profile was observed during the single-strain omission when Lacticaseibacillus sp., Lactiplantibacillus sp., and Levilactibacillus sp. were omitted, than with the complete culture fermentation. Specifically, a monoculture of Lactococcus sp. and its presence in a mixed culture delivered increased levels of diacetyl and acetoin compared with the presence of *Lacticaseibacillus* sp. either alone or in the multi-culture. In addition, the omission of Lactococcus sp. exhibited lower levels of 2-nonanol, phenylethyl alcohol, 1-butanol, 3-methyl, acetate, and 1-butanol, 3-methyl. The compounds 3-methyl butanal, diacetyl, and acetoin are known to be odour-active compounds in mabisi and impart a nutty, creamy, and buttery aroma to the product (Chapter 3 of this thesis). In contrast to *Lacticaseibacillus* sp. and *Lactococcus* sp., *Lactiplantibacillus* sp. was found to be associated with lower levels of acetic acid, acetoin, nonanoic and benzyl alcohol, and the absence of diacetyl. On the other hand, Levilactibacillus sp. had low levels of benzyl alcohol, acetoin, and butanal, 3- methyl while the other compounds were mostly absent. Despite their seemingly negligible contribution to the acidification of milk, the role of yeast in aroma formation was quite evident. HS-SPME-GC/MC results provided useful information about the metabolism of the yeast species used in the mixed culture, which was marked by increased levels of esters in the presence of *Candida* sp.

The species of *Lacticaseibacillus* sp., *Lactococcus* sp., and *Candida* sp. demonstrated the greatest potential as starter cultures in fermented dairy products. The overall quality of the final products can be considered a sum of the metabolic activity of all five strains, despite the minimal impact of *Lactiplantibacillus* sp. and *Levilactibacillus* sp. Further investigation is needed to ascertain the functional significance of *Lactiplantibacillus* sp. and *Levilactibacillus* sp. in a starter culture. Additionally, evaluating all the strains for stability and susceptibility to pathogenic invasion is essential.

Evaluating the strains based on their acidification capacity and volatile compound formation is only an initial step in the selection of microbes for starter cultures, as these strains may not adapt sufficiently to all production conditions to achieve the desired properties and functionality of the end product. Traditionally, fermented products are typically rather versatile and robust in adapting to environmental changes. Therefore, it is crucial to thoroughly and extensively characterize the strains, determine their functional characteristics, and also assess their viability during storage. This includes investigating their ability to maintain consistent acidification rates, flavour profiles, and microbial composition over extended periods. Understanding how starter cultures interact with other microbes and environmental factors will help optimize their use, ensuring the production of high-quality, safe, and authentic fermented dairy products that meet consumer expectations. Moreover, LAB and yeasts are known to produce various vitamins during fermentation, and some of them may have probiotic potential that needs to be explored. Therefore, selecting appropriate strains with these characteristics can enhance the nutritional and health outcomes of fermented milk (Leroy & De Vuyst, 2004). Besides, these strains may also possess other functional properties, such as the ability to produce exopolysaccharides and bacteriocins, which are crucial for the food industry. Exopolysaccharides can contribute to the texture and viscosity of fermented dairy products and consequently, enhance their stability and quality, while bacteriocins are antimicrobial peptides

that can inhibit the growth of spoilage and pathogenic microbes and ensure food safety (De Vuyst & Degeest, 1999; Gálvez et al., 2007; Gänzle, 2015; Leroy & De Vuyst, 2004; Sawadogo-Lingani et al., 2010).

Generally, the results indicated the potential of the isolated strains to be used for starter cultures to mimic the characteristic profile of traditionally fermented mabisi. In an experiment to compare starter-made mabisi and two variants of traditional mabisi (Barotse and Tonga mabisi), the aroma profile of the starter-made product was comparable to the traditional variant (Fig. S2). This could be pursued further with concrete experiments.

5.0 Conclusion

The results of this study highlight that traditional fermentation offers a valuable resource of strains for starter culture development. The findings provide insights into the role of indigenous LAB and yeast strains in shaping the distinctive flavours of fermented dairy products. Among the LAB strains, *Lacticaseibacillus* sp. and *Lactococcus* sp. species were notable for their strong acidification potential and significant contribution to the volatile profile. They also performed well in mixed culture fermentation, showing their promising potential for further application in dairy fermentation. Yeast played a unique role by imparting distinct volatile compounds to the fermented milk.

Acknowledgments

We are grateful to the Integrated Research Fund (INREF) at Wageningen University and Research for the financial support towards this research.

Supplementary material

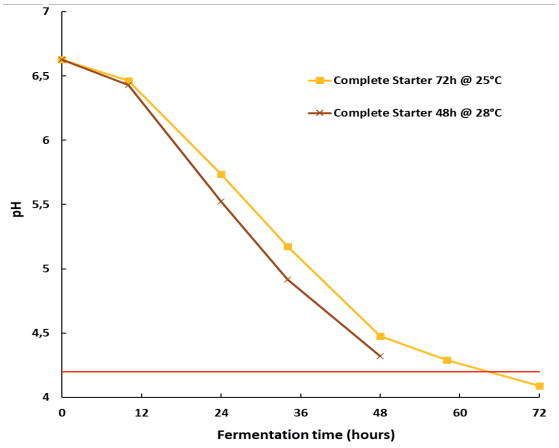

Fig. S1. Comparison of the acidification capacity (pH) of the complete starter culture during fermentation at 28 °C for 48 hours and 25 °C for 72 hours. The desired final pH is indicated by the red horizontal line and was only achieved after 65 hours of fermentation at 25 °C.

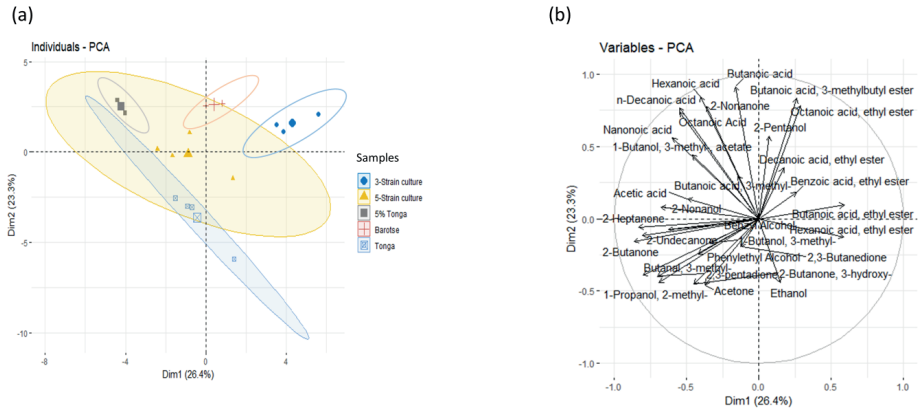

Fig. S2. a) PCA plot projection of the complete starter culture, UHT milk inoculated with 5% Tonga mabisi, traditional Tonga, and Barotse, based on the normalized peak area percentages of the volatile organic compounds.

References

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CHAPTER

General discussion

Thelma W. Sikombe

1.0 Scope and main findings of this thesis

Traditional food fermentation has long been an integral part of rural community food systems, serving as a means of food preservation, providing essential nutrition, and offering an economic source of income (Aworh, 2008; Holzapfel, 2002). In the industrialized world, food fermentation generally relies upon the addition of well-defined starter cultures to raw materials (Parente et al., 2004), fermentation in low- and middle-income countries is an artisanal activity largely performed through spontaneous processes. This means that the outcome of these fermentation processes depends on the presence and activity of microbes from the environment and the microbiota associated with the raw materials as inoculum. This thesis centered around how traditional fermented foods could be further optimized.

In this thesis, I specifically focussed on the production of mabisi, a traditional fermented dairy product from Zambia. Mabisi holds a significant cultural and nutritional essence, forming a vital component of the local diets. Mabisi is revered among the locals for its rich heritage and distinct organoleptic properties. Traditional fermentation practice has been used from time immemorial, and in the Zambian context, no recorded cases of foodborne illnesses have been associated with mabisi consumption. This long history of safe consumption highlights the potential protective benefits of traditional fermentation methods, such as the production of organic acids and other antimicrobial compounds that prevent the growth of harmful pathogens. This further suggests that despite being produced under non-commercial conditions, these practices have successfully ensured the safety of the products for generations. However, spontaneous fermentation may yield unpredictable product outcomes, compromise product safety and quality, or result in failed fermentation (Holzapfel, 2002). Furthermore, a recent study by Schoustra and colleagues (2022) suggests that the current traditional methods of mabisi production using raw milk lead to a microbiologically safe product, although some potential pathogens may approach critical limits.

Product optimization is essential for improving the small-scale traditional industry as it motivates small-scale farmers, in this case, the local dairy farmer to increase the production of raw materials (Sanni, 1993). Optimization is necessary for establishing the best process conditions for upscaled production and delivery of safe, more sustainable, and better-quality fermented foods. Optimizing the production of mabisi will offer opportunities for consumers to consistently enjoy the many benefits of fermentation including health and nutrition and provide economic empowerment to the local producers (Materia et al., 2021). Previous research by Moonga and colleagues (2019) inspired part of the research of this thesis. They described different production methods, identified key production parameters, and analyzed the microbial community composition of different traditional mabisi products from across the country. They demonstrated variations in the microbial community composition and consequently the volatile compounds and product texture such as thickness upon fermentation at different temperatures depicting seasonal variations in Zambia (Moonga et al., 2021). Due to the uncontrolled nature of the current production of traditional mabisi, the process has many variables, such as the raw milk, environmental conditions, and the fermenting containers, which means variable communities of fermenting microbes and ultimately the final product. Consequently, without optimizing the fermentation parameters, the consistent delivery of product benefits to the consumer cannot be guaranteed. Additionally, the expected stability and shelf life of the product during storage may be compromised. Building on this previous work (Moonga et al., 2019; Moonga et al., 2021; Moonga et al., 2020), this thesis examined key mabisi aspects essential for optimizing production: the characteristics of mabisi including sensory and consumer perception, shelf life at varied temperatures and potential of autochthonous microbial strains for use as starter cultures. This is essential to provide a more scientific basis for optimized and upscaled production.

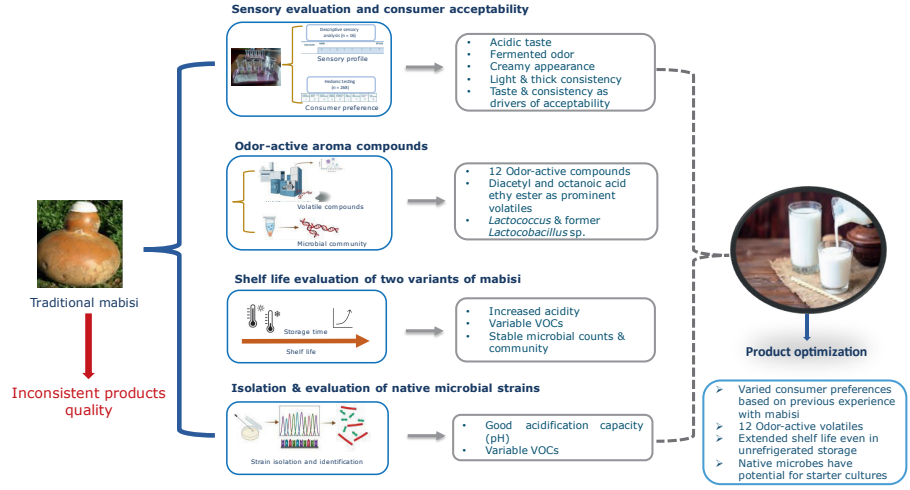


Fig.1. Schematic presentation of the thesis chapters. This schematic provides an an overview of the research activities and outcomes described in this thesis.

Figure 1 outlines the scope and main findings of this thesis work. Specifically, using appropriate sensory tools we described the sensory profile and assessed the organoleptic attributes influencing consumer perception of four widely produced variants of traditional mabisi namely; backslopping, barotse, illa, and tonga mabisi (Chapter 2). Using detection methods that distinguish specific volatile compounds perceived by human olfactory senses, we identified key aroma compounds that constitute each of the mabisi variants. We also identified the microbial community associated with the detected volatile compounds (Chapter 3). We further studied the effects of storage temperature on the quality and microbial dynamics and found that the shelf life of mabisi extends to over two weeks under ambient storage conditions (Chapter 4). Finally, we isolated the autochthonous microbes to test their practical application as mabisi starter cultures that could be employed to promote controlled fermentation leading to predictable outcomes (Chapter 5). Investigating these aspects of mabisi processing gave an in-depth understanding of this traditional dairy product, highlighting its unique characteristics

and opportunities for improvement and optimization. Through this systematic approach, this thesis provides valuable insights into consumer preferences, key odor-active compounds, shelf stability, and the potential of the native microbial resources of mabisi as starter cultures.

The findings of this thesis are crucial for augmenting efforts to optimize the production of mabisi, ensuring consistent product quality and safety, and thus building consumer confidence and increasing the market demand for this traditional product. In addition, the results can be a useful reference for similar studies on other traditional fermented foods globally, thereby promoting cross-cultural knowledge exchange in the field. In this general discussion, I now further contextualize each of the main findings, followed by a synthesis towards optimization of production of mabisi. I further outline generalities towards optimization of traditional fermented foods in general and the impact this may have on sustainable development.

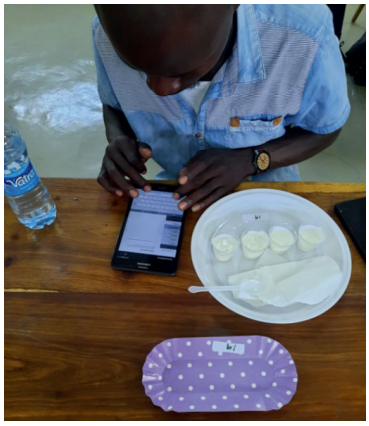
2.0 The sensory appeal and consumer perception of mabisi for product optimization and improvement

The sensory evaluation and acceptability of mabisi by consumers was the first systematic study to profile these characteristics of the product (Chapter 2). Using a modified quantitative descriptive analysis, a panel of judges identified and evaluated the intensity of relevant descriptive terms to understand the attributes that are important for overall product acceptance. Mabisi consumers from three provinces of Zambia representing urban and rural consumers provided their preferences for four variants of mabisi products. Significant differences were observed among the four variants of mabisi for all the described sensory attributes except the fermented odor. Product thickness was the distinguishing attribute of the products, and together with a creamy consistency, these were the drivers that determined consumers' acceptance.

Given the rich and intense fermented aroma of mabisi, we decided to explore and identify the volatile compounds eliciting these odors. Chapter 3 provides insights into the volatile

compounds that give mabisi its characteristic aromas. This knowledge is essential for customizing aroma profiles and the quality of mabisi to correspond to the preferences of different consumer segments. Although odor was not a distinguishing attribute among the variants during the sensory evaluation of mabisi (Chapter 2), variations existed in the odor-active aroma compounds contributing to the aromas of each variant (Chapter 3). The inconsistency in the findings may be explained by several factors including the effect of synergistic interactions among the odors, causing some odor-active compounds to mask the aromas of others, resulting in a similar perceived odor by consumers even when the specific compounds involved were different (Blank, 1996). Likewise, certain compounds may amplify each other's odor, creating a similar overall aroma profile despite individual differences between compounds. Moreover, human olfaction is complex and can coalesce multiple odor-active compounds into a singular perception, levelling out the differences in individual volatile compounds (Brattoli et al., 2013.

Leveraging sensory characteristics and particularly consumer feedback is useful for understanding consumer perception and can be applied to optimize production, making necessary adjustments to improve the quality, appeal, and consistency of mabisi. This is also useful in ensuring consistent production of desirable outcomes and reproducibility on a larger scale without compromising the unique mabisi characteristics.

a)  b) 

c) 

Fig. 2. Sensory evaluation of different mabisi products with consumers from different districts in Zambia. Consumer sensory evaluation was carried out using Central Location Testing in Southern Province, Lusaka, and Western Province. (a) Briefing consumers in Kalingalinga, Lusaka about the study and the procedure for the sensory evaluation of the mabisi samples, (b) Consumer evaluating the samples in Choma, (c) Meeting the residents of Lealui in Mongu in preparation for the consumer acceptability study.

3.0 Shelflife, a key attribute for product quality and safety

Food security is a main motivation for traditional fermentation at household level as fermentation presents an easily applicable and cheap means of preserving foods in situations

where refrigeration is unavailable (Oyewole, 1997). It is also a means of dietary diversification in most resource-limited settings, particularly during the seasons of lack when access to fresh and highly perishable foods is restricted. While commercially manufactured fermented foods are generally produced under controlled environments with Good Manufacturing Practices (GMP) in place, traditional fermented foods are normally made in uncontrolled conditions with sometimes questionable hygiene practices. Coupled with the lack of proper low-temperature storage facilities, this presents an issue of concern for household-level and small-scale food processors in rural Zambia. This situation is further exacerbated by the warm climatic conditions prevalent in the country. The high temperatures create a conducive environment for the growth of unwanted microorganisms, making it more difficult to preserve food safely. Thus, maintaining the quality and safety of food becomes a challenge, often leading to increased food waste and reduced access to fresh, nutritious options. These conditions emphasize the importance of effective preservation methods in mitigating the effects of warm climates on food security. Fortunately, fermentation does not just extend the shelf life of foods and avert losses, but it also reinforces product safety through the anti-microbial properties exhibited by the lactic acid bacteria through the production of various compounds such as organic acids, hydrogen peroxide, diacetyl, and bacteriocins (El-Ghaish et al., 2011; Schoustra et al., 2022).

In this thesis work, we used mabisi as an archetypical model of how temperature influences certain product characteristics during the storage of traditional fermented dairy products (Chapter 4). The effect of temperature on the pH was observed towards the end of un-chilled storage with evidence of post-acidification as the pH dropped to a low but safe range to inhibit the growth of pathogens and comparable to other fermented foods (Beukes et al., 2001; Schoustra et al., 2013). However, only limited post-acidification was observed during chilled storage in a refrigerator, indicating product stability, and highlighting the ability of low

temperatures to promote product resilience. Similarly, the influence of temperature on the microbial population was generally minimal and not significant during chilled storage. While a significant temperature effect was reflected in product pH and titratable acidity (TTA), bacterial community stability was maintained during storage, irrespective of temperature up to the end of storage. This suggests that despite the shift in the functional properties, traditional mabisi remains relatively stable regarding the bacterial community during both the ambient and refrigeration storage. The stability and robustness of traditional mabisi are credited to its complex microbial ecosystem dominated primarily by lactic acid bacteria (LAB) and complemented with yeasts. Nonetheless, refrigerated storage is recommended for preserving the desired qualities.

The findings of this study highlight the resilience of the natural and complex microbial communities of traditional fermented products. Specifically, this demonstrates that milk-based traditional fermented products exhibit a high degree of stability against temperature variations over a storage period of upto 24 days. This further illustrates the significance of fermented food systems in preserving product quality, and this improves further if production is performed in conjunction with GMP. The resilience of mabisi has also been demonstrated by studies on pathogenic invasion during the fermentation process (Schoustra et al., 2022). Nevertheless, the fermentation temperature and time are critical here due to their influence on the acidification rate which is important to provide a hostile environment to undesirable microbes that cause spoilage and that could be pathogenic. Additionally, identifying the chemical degradation and formation of harmful compounds during storage and understanding their impact on product quality, safety, and shelf life, could be a key focus for future research. This could potentially involve studying the changes in sensory attributes, nutritional composition, and the overall safety profile of the products over time, leading to improved storage techniques and product stability.

4.0 Starter cultures to improve traditional fermentation

Currently, traditional mabisi production relies on spontaneous fermentation. However, in industrial settings, the use of starter cultures in fermentation processes has proven to be an effective alternative (Holzapfel, 2002). Starter cultures promote controlled fermentation and ensure a more predictable final product. With the call to upscale production to serve the growing consumer demands and maximize the benefits of fermented products, isolating, identifying, and characterizing specific microbial strains involved in the fermentation processes could facilitate the development of tailor-made cultures and improve the production of mabisi. Starter cultures have been used to initiate the fermentation process, produce lactic acid, and bring about the desired quality of the final product. They are essential for optimizing processes, allowing for controlled fermentation, and yielding predictable outcomes (Holzapfel, 1997; Parente et al., 2017). Using starter cultures also builds consumer confidence in the hygienic standards applied during fermentation.

In Chapter 5, the potential of the microbial resource of mabisi was determined based on the acidification capacity and volatile organic compound production using mono and mixed cultures of the isolated strains. This also helped determine the contribution of each isolate to the fermentation process. The isolated microbes comprised the dominant bacterial species and the less dominant yeasts previously reported in mabisi (Moonga et al., 2020; Schoustra et al., 2013). The species included the bacteria genera *Enterococcus* sp., *Lacticaseibacillus* sp., *Lactiplantibacillus* sp., *Levilactibacillus* sp., and *Lactococcus* sp., and the yeast, *Wickerhamiella* sp. and *Candida* sp. During the trial fermentation with UHT milk, pH was found to drop to safe levels of 4.2 with *Lactocaseibacillus* sp. and *Lactococcus* sp. showing the greatest acidifying and both *Lactococcus* sp. and yeast (*Candida* sp.) contributing to the unique volatile organic compounds of the final product. Overall, the isolates exhibited potential for

use as starter cultures. However, further research is warranted to carefully select and characterize isolates with specific desirable qualities. Microbial strains contributing to the sensory characteristics and producing compounds with health and nutritional benefits such as vitamins and probiotics should be thoroughly studied. More specifically, the impact of defined mixed starter cultures on product characteristics such as consumer acceptability, food safety, and the health benefits of mabisi should be explored. Moreover, the robustness of the starter culture in the fermentation system must be tested to ascertain the resistance to contamination and understand the performance in an upscaled scenario (Gong et al., 2017). Similarly, in line with the findings of Chapter 4, the shelf life of the starter-made mabisi is another important aspect that requires focus and further investigation. With the possibility of applying starter cultures from the mabisi indigenous microbes, conducting a sensory evaluation and getting consumer feedback on the starter-produced mabisi would be an appropriate way of determining the efficiency of starter cultures.

Our findings can translate to improving the production of mabisi and other traditional fermentation systems using autochthonous microbes and promote greater control over the process for consistent product quality. Commercially produced starter-based mabisi is already available in the Zambian formal markets from various dairy manufacturers. However, this version of mabisi is primarily made using only two commercial milk strains, and its flavor is often said to lack the distinctive, rich appeal of "traditional mabisi".

5.0 The potential of upscaling traditional mabisi production

The government of Zambia supports the dairy sector as a vehicle for economic growth and poverty reduction among the rural poor and smallholder farmers (Mumba et al., 2011). Smallholder dairy farmers play an important role in the Zambian dairy sector, providing about 45% of the country's milk production (Mumba et al., 2011). About half of this milk is sold to

large commercial processing companies through the Milk Collection Centres (MCC) owned by the dairy cooperatives dotted around the country. The remaining half is either used to make mabisi at the MCC or is sold to the local communities for home consumption or household mabisi production. Household mabisi production is typically carried out by women, who depend on it for their livelihoods (Materia et al., 2021). The production and sale of traditional mabisi provide these local women producers with financial independence and the means to support their families. Beyond economic benefits, the art of mabisi production is crucial in preserving the traditional heritage and passing on indigenous knowledge through generations. Upscaling the production process of traditional mabisi is essential for avoiding any losses of milk and encouraging more production to promote rural economic development (Adesulu & Awojobi, 2014; Materia et al., 2021). There are several benefits to upscaling traditional fermentation processes, each contributing to the demand for larger-scale and more efficient production. More specifically, upscaling will improve the production scale of traditional mabisi and promote availability to meet the growing demand for these nutritious foods. It also promises to deliver more consistent quality and enhance sustainable production for dietary diversity and income generation.

Zambia has set standards and defined criteria for specific foods sold on the market including milk and milk products. Before food products are placed on the formal Zambian market, they have to be certified as compliant with the food safety and quality regulatory requirements. Currently, there are no such standards and specifications available for traditional mabisi. In the absence of these product standards and specifications, small-scale and traditional mabisi producers cannot fully benefit from their enterprises as they are unable to reach the formal markets and thus a wide consumer base (Schoustra, 2024). Mabisi producers miss out on maximizing their income due to untapped sales opportunities. The standards specify that food must be stored, transported, packaged, and labeled appropriately to maintain its quality and

stability. Adherence to these standards helps keep the food safe and of high quality from production to consumption. Moreover, it promotes transparency and traceability in the food value chain, which are crucial for fostering a healthier, and more sustainable future.

a)  b) 

Fig. 3. (a) A Milk Collection Centre (MCC) in Choma, Zambia. (b) Farmers delivering milk at the MCC. MCCs are centralized points that play an important role in the dairy value chain, particularly in the rural and peri-urban areas in Africa where small-scale dairy farmers bring their raw milk for collection.

Building on the Zambia Bureau of Standards (ZABS) recently introduced approach of legalizing traditional processing through the implementation of a code of practice, the findings of our study and previous research provide valuable information to support traditional mabisi production (Moonga et al., 2021; Schoustra, 2024; Schoustra et al., 2022). This information can be used to define key processing parameters necessary for optimizing, upscaling, and standardizing mabisi production enabling small-scale and traditional producers to access the formal market systems. This will also increase the demand for raw milk, provide sustainable incomes, and ensure food security for small-scale dairy farmers and all the actors of the

traditional value chain. Similarly, more consumers can diversify their diets and improve their nutritional status through the consumption of the nutrient-rich mabisi.

Currently, small-scale traditional producers of mabisi typically have a production capacity that ranges from 5 to 20 litres. However, with process optimization and improved market access, this capacity could potentially increase to 500 litres with minimal technological requirements (Schoustra, 2024).

6.0 Meeting the demands of consumers

The significance of the gut microbiome in supporting the immune system, and knowledge of the link between the consumption of fermented foods and gut health, have boosted the popularity of traditional fermented foods in the last decade (Szutowska, 2020; Xing et al., 2023). Consequently, the perception of the health benefits of fermented foods has substantially increased, particularly the urban consumers. Similarly, the growing emphasis on proactive health maintenance among consumers, as opposed to waiting for illnesses to occur, has led to a cultural shift towards preventive health measures. This cultural shift is a major driving force behind the increased interest in fermented foods. The positive strides already made toward healthy eating must be leveraged further to inspire a universal transition of the food systems. Without the consumer, there can be no motivation for the production of healthy foods.

In the Zambian context, the current consumer demand and interest for these products have resulted in the reinvention of traditional fermentations, as evidenced by the presence of commercially produced version of mabisi on the market by established manufacturers. If optimized and upscaled, traditional mabisi fermentation has the potential to meet the growing demands of consumers through increased volumes.

To get in-depth insights into consumer behavior, sociological aspects were also included in the consumer acceptability survey interviews by asking questions regarding their attitude towards mabisi such as frequency of consumption, preferred type of mabisi (commercial or traditional), and the reasons behind their preferences (Chapter 2). This information is essential to guide the improvement of indigenous knowledge and processes around mabisi to align with consumer needs and create targeted messages for its promotion. Concerning the consumption frequency pattern, 43% of consumers indicated that they consume mabisi 3 times a week, 24% said they consume mabisi at least once a week and only 17% consume it occasionally (Fig. 4a). Most consumers preferred the traditional mabisi over its commercial counterpart, citing the better taste, unique and intense flavors as the main motivation for their preference. Other reasons behind the preference for traditional mabisi included the availability of the product in their communities and affordability in comparison to the commercial brand. The preference of traditional mabisi over the commercial brand was also observed previously (van de Ven, 2018). Commercial mabisi costs about three times as much as the traditional brand, which many consumers especially in rural communities can not afford. Those who prefer the commercial brand over the traditional brand attribute their preference mostly to the fact that the commercial brand is produced in a hygienic environment thus assuring its safety. Overall, 61% of consumers had no preference between traditional or commercial mabisi, while 25% preferred traditional mabisi, and only 14% preferred the commercial type of mabisi (Fig. 4b).

Despite the proportion of consumers with a preference for both commercial and traditional mabisi being greater than that of consumers whose preference was for either, the older consumers generally preferred the traditional brand of mabisi over the commercial one (Fig. 5). This could be attributed to the fact that traditional mabisi is perceived to have more intense flavors compared to the commercial brand and older individuals generally have higher perception thresholds for basic tastes like sweetness, saltiness, bitterness, and acids. Moreover,

older adults may often experience deteriorated sensory perception, resulting from age-related health issues (Baugreet et al., 2017; Liu et al., 2022).

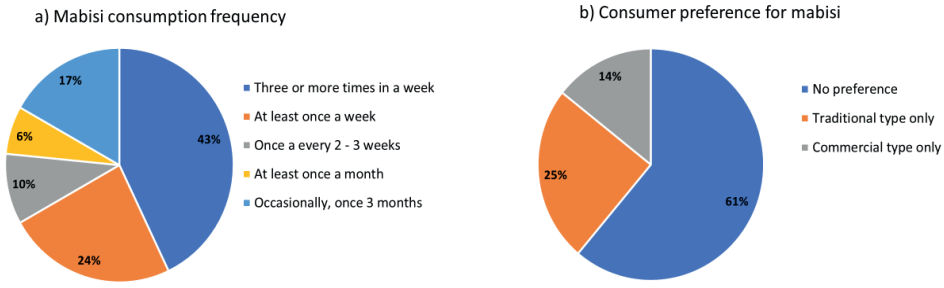


Fig. 4. Insights on mabisi consumption and preferences among consumers: (a) Mabisi consumption frequency patterns and (b) Consumer preference for traditional and commercially produced mabisi.

While consumers who preferred commercial mabisi were more concerned about product safety, those with a high preference for the traditional brand prioritized the sensory appeal over its safety. The consumers were generally aware that mabisi either commercially or traditionally produced offers nutritional and health-promoting benefits but largely based their preference patterns on other product characteristics as earlier alluded, such as, flavour and texture. Since traditional mabisi is an artisanal production, prepared at the household level, its safety is entirely dependent on the level of hygiene applied by the producer. Moreover, the lack of quality assurance enforcement by regulatory bodies for such products has led to many consumers losing confidence in their safety.

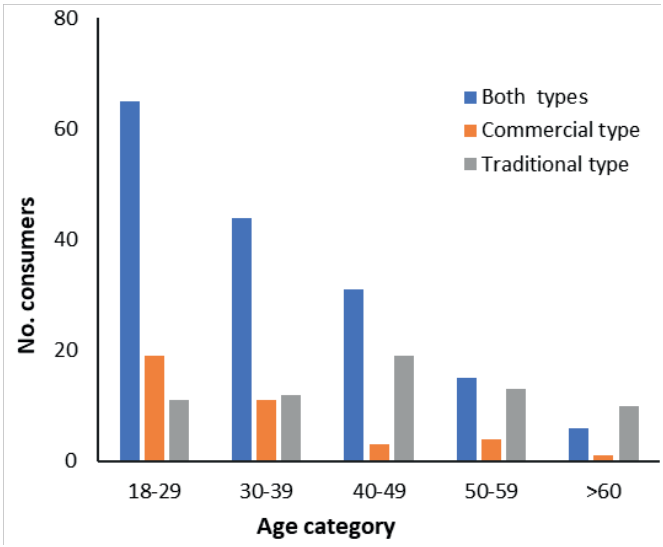


Fig. 5 Preference patterns for the traditional and commercial brands of mabisi across the different age groups. Older consumers preferred the traditional brand more than younger consumers.

7.0 Batch-to-batch variations and quality control

Natural fermentation processes such as traditional mabisi fermentation support increased microbial diversity as influenced by the raw milk, climatic conditions, producer hygiene, and fermentation containers (Holzapfel, 2002). This is reflected in the batch-to batch variations in quality and safety of the final product. In a formal market system, it is difficult to maintain a loyal consumer base with inconsistent quality as consumers expect a reliable and consistent product at every purchase. Inconsistent quality may be viewed negatively by consumers and it gives an impression of insufficient quality assurance measures which destroys trust in the product and thus makes access to the wider market difficult (Zhang et al., 2014). Moreover, product inconsistency will make meeting regulatory and quality control requirements challenging resulting in compliance issues.

Optimizing the production of traditional fermentation procedures is crucial for establishing the best process conditions for upscaled production and delivery of safe, more sustainable, and better-quality fermented foods. To effectively upscale the production of traditional mabisi, one of the key issues is overcoming the challenges of recurring batch-to-batch variations in product quality (Xing et al., 2023). Optimized production processes can help gain control of the fermentation process and minimize variations in product quality. Consequently, more superior and consistent outcomes in nutritional, health, and sensory properties are expected thus allowing the product to deliver its intended benefits to the consumer. This is crucial for successfully placing traditional mabisi on the formal markets and for the product to meet regulatory specifications. Consistent product quality entails that consumers can confidently rely upon the safety, health, and nutritional benefits and enjoy a consistent sensory experience.

8.0 Sustainability and food security

The rising demand for traditional fermented foods can stimulate small-scale enterprises, create demand for raw milk, more job opportunities, and foster economic growth, particularly in rural communities where dairy farming and traditional fermentation practices are widespread (Marshall & Mejia, 2011; Materia et al., 2021). This growth will enhance food security and nutrition by making nutritious traditional foods more widely available and supporting sustainable agricultural practices. This can reduce the need for rural-urban migration and alleviate related social challenges, such as urban crowding and the strain on urban infrastructure. By generating employment, fermentation has the potential to alleviate poverty levels among rural and peri-urban populations (Aworh, 2008). The local mabisi processors mostly women and small-scale dairy farmers in rural areas are empowered with additional streams of income to improve their livelihoods and household food security.

My thesis has specifically demonstrated that meeting the needs of local consumers requires offering diverse mabisi variants tailored to their preferences (Chapter 2). During my field studies, it was observed that local processors are capable of using different traditional techniques in their fermentation practice to produce variations of mabisi and this was also recorded in the previous studies on mabisi (Moonga et al., 2019). The many variants of mabisi have distinct sensory characteristics and consumer preference for each varies largely based on their previous experience with the product.

The mabisi variants, especially the four described in Chapter 2, have the potential to be further optimized and upgraded to introduce a broader selection of traditional mabisi products in formal markets. By doing so, these variants can cater to the diverse tastes and preferences of different consumer segments, ensuring that the cultural heritage of mabisi is preserved while also appealing to a wider consumer base. Moreover, each mabisi variant may offer distinct health and nutritional benefits, providing consumers with a range of options that meet their dietary needs and preferences. This diversification not only enriches the consumer experience but also opens up new opportunities for promoting the unique qualities of mabisi as both a nutritious and culturally significant food product

Further, in Chapter 4, I demonstrated that the shelf life of traditional mabisi is well over two weeks even without the need of refrigeration. This allows mabisi to be transported over relatively long distances, where it could reach urban areas. The consumer survey and earlier work on mabisi highlighted that consumers in urban areas prefer the traditional version of mabisi over the commercial one just like their rural counterparts. However, the limited availability of traditional mabisi in urban areas humpers its wide consumption, and consumers settle for what is available. The prolonged shelflife thus provides a viable opportunity to connect urban consumers to the traditional processors and their cultural heritage. Bringing the

traditional mabisi to the urban market is key to increasing the incomes of traditional mabisi processors who are mostly domiciled in rural areas where milk production is readily available. This supports the government of Zambia's agenda of economic development and job creation to reduce levels of poverty of in the country. This highlights the potential of mabisi to improve the livelihoods of rural communities while meeting the nutritional needs of both rural and urban consumers.

Finally, the findings reported in Chapter 5 that starter cultures could be developed for traditional mabisi is potentially of use to local dairy cooperatives that currently use starter cultures to produce global yogurt and have expressed interest to rather produce mabisi should a starter culture exist. This would undoubtedly be an improvement and option to control and upgrade the existing traditional fermentation technologies for predictable product outcomes (Holzapfel, 2002). Considering the consumer preference for mabisi with a rich aroma and flavor typical of traditional version, mixed-strain cultures would be the appropriate cultures for use. Single strain cultures produce a narrower spectrum of volatile compounds compared to mixed-strain cultures that have a broader range of compounds due to their synegistic interactions and metabolic activities of the different microbes. This consequently leads to the development of more complex volatile profiles for the mixed strain culture (Chapter 5) (Sarhir et al., 2023).

Since traditional fermentation utilizes locally available raw materials, it is a cost-efficient process that promotes sustainable rural development through improved food and nutritional security. As the world faces an unprecedented food crisis, many people's lives are at risk of acute food insecurity. Traditional fermentation for rural African communities can create opportunities to influence resilience in the food systems and align with the broader sustainable

development goals by supporting sustainable food production systems. This is achieved in several ways (Akinsemolu, 2018);

- Fermentation has been used to preserve and extend the shelf life of perishable products, thus reducing food waste and contributing to SDG12 on responsible consumption and production.
- By preserving and enhancing the nutritional value of foods, traditional fermentation aids in ensuring household food security and access to nutritious foods contributing to SDG2 on zero hunger.
- Fermented foods with their beneficial compounds promoting gut health and overall well-being contribute to SDG 3 on good health and well-being.
- As small-scale and household-level producers carry out traditional fermentation practices, it's a job creation avenue for many rural and peri-urban inhabitants thus promoting economic growth and reducing poverty (SDG 1 & 8)(Anukam & Reid, 2009).
- The use of local and indigenous raw materials for fermentation supports biodiversity and sustainable land use practices contributing to SDG 15 on Life on Land.

9.0 Concluding remarks and future perspectives

Traditional fermented foods play an important economic role in the growth of rural communities by contributing to the nutritional requirements of consumers and generating income for the local producers. The potential of fermented foods as a method for food preservation and value addition is crucial for enhancing rural food systems.

Given the benefits of traditional fermented foods, it is essential to improve and upscale production to make them accessible to a broader consumer market. However, upscaling and maximizing this potential entails overcoming the challenges of batch-to-batch variations and

unpredictable quality, microbial process control, shelf life and stability, safety and hygiene, and regulatory compliance which requires collaborative efforts from a diverse range of stakeholders including the scientific community, regulatory bodies, and the local producers and consumers. Aspects concerning product characteristics such as the sensory and olfactory profile, the drivers of consumer acceptability, the shelf life as well as the microbial resources with potential for application as starter cultures have been tackled in this thesis using traditional mabisi. The outcomes of this research are expected to provide valuable insights addressing the knowledge gaps regarding the characteristics of traditional fermented foods. This information aims to enhance the production dynamics while preserving the unique functional attributes of mabisi. Nonetheless, further detailed research on the characterization of the microbes isolated from mabisi would be another research focus. This includes identifying specific strains, probably a whole gene sequencing rather than the 16S rRNA sequencing to provide comprehensive data for precise identification. In additional, understanding the roles of the strains in the fermentation process, and evaluating their contributions to the safety, flavor, and nutritional profile of the final product. Finally, more information on their robustness in the fermentation system is also another research direction to be considered. With the knowledge of microbial communities, we can improve the consistency and quality of traditional fermented foods and increase the competitiveness of mabisi on the market.

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General summary

Traditional fermentation is one of the oldest processing techniques that has been used for centuries and remains relevant to preventing major food losses in many regions across the world. Adopting traditional fermentation at a large scale presents many opportunities to improve the future prospects of foods and support the development of sustainable food systems. Traditional fermentation is typically driven by spontaneous processes that are employed in many resource-limited communities as an affordable preservation method for extended shelf life of food and improved quality and safety. The fermentation processes are initiated by the diverse and mixed microbial communities present in the raw materials and in the environment. The diversity of the microbial community is influenced by the prevailing environmental conditions such as the temperature, production method, type of container, and geographic location, which play an important role in driving productivity and functionality. Specifically, the prevailing environmental conditions influence microbial metabolism, enzymatic activity, and the interaction among the different species which leads to the creation of a rich and varied mixture of metabolites that define the characteristics and quality of the final product. However, because fermentation conditions are uncontrolled, batch-to-batch outcomes are largely variable and may lead to inconsistent product quality.

There has been an upsurge of interest in (traditional) fermented food production over the last few decades due to the perceived health benefits of these foods. In Zambia, traditional fermentation of mabisi, a dairy product made with raw milk, is largely an artisanal activity practiced by women in rural areas to supplement household incomes. With the growing interest and consumer demand, mabisi has shown the potential to significantly enhance the livelihoods of local producers and contribute to rural development. However, its current production methods (and volumes) hinder access to formal markets, as the product may not comply with

food quality standards and regulatory requirements. This underscores the need for optimization and upscaling of the production of mabisi to maximize its potential.

In this thesis, we studied mabisi to gain a comprehensive understanding of its characteristics, from its sensory properties and consumer perceptions to the key volatile compounds that influence its unique and rich aroma profile, which consumers find so appealing (Chapters 2 and 3). Using modified quantitative descriptive analysis, a semi-trained panel of assessors generated important organoleptic terms describing the sensory profile of mabisi. Preference for the mabisi variants differed widely among the consumers though the thick and creamy texture were the main drivers for their acceptability. In Chapter 3, twelve key odour active compounds were identified using GC-O analysis. *Lactococcus* sp. and the former *Lactobacillus* sp. were highly correlated with the most common volatiles identified; diacetyl, and the esters that included ethyl butanoic acid and ethyl octanoic acid. These findings are significant in identifying the distinct VOCs that set these traditional products apart when production methods differ. This could be leveraged to improve and create different variants of mabisi tailored to various consumer segments.

In Chapter 4, we assessed the properties of mabisi that influence the shelf life when stored at ambient and refrigeration temperatures over a period of two and three weeks respectively. Temperature selection was based on typical storage practices among mabisi producers and consumers.

Despite a decrease in pH and an increase in TTA, after day 12 the microbial population and the community remained stable for most of the storage period. This study highlighted that mabisi can remain shelf-stable for over 14 days without refrigeration and the two different Mabisi variants investigated exhibited no differences in their shelf-life. This information is crucial for

expanding mabisi production, particularly when considering transportation from rural areas to urban markets to reach a wider consumer base.

Although starter cultures are available for many commercial fermentations, traditional fermentation is dependent on spontaneous processes, hence leading to varied and inconsistent product outcomes. The use of starter cultures can ensure final products that are microbiologically safe with consistent sensory and quality characteristics. In Chapter 5 we isolated and identified the autochthonous microbes from traditional mabisi to examine their suitability for application as starter cultures. Various microbial species were isolated and identified including, *Enterococcus* sp., *Lactocaseibacillus* sp., *Lactiplantibacillus* sp., *Levilactibacillus* sp., and *Lactococcus* sp. and two yeast species of *Wickerhamiella* sp. and *Candida* sp. From the isolated strains, four bacteria (*Lacticaseibacillus* sp., *Lactococcus* sp., *Levilactobacillus* sp., *Lactiplantibacillus* sp.), and one yeast strain (*Candida* sp.), were evaluated both as mono- and in mixed-strain cultures in fermentation trials with milk. The *Lactocaseibacillus* sp. and *Lactococcus* sp. exhibited a great acidifying potential, while both *Lactococcus* sp. and yeast (*Candida* sp.) also contributed unique volatile organic compounds to the final product. Overall, this chapter demonstrated the potential of the isolated strains to be used for starter cultures in mabisi production.

The government of Zambia envisages a well-nourished and healthy population by 2030 and identifies food security as one of the critical elements of the country's economic development. Therefore, understanding the characteristics of traditional fermented products like mabisi and scaling up their production has potential to improve the country's food security and nutrition by increasing the availability of nutritious, culturally significant foods. These traditional foods can serve as affordable, nutrient-rich options that contribute to dietary diversity and better health outcomes, particularly for low-income communities. Additionally, expanding the

production of traditional mabisi supports sustainable agricultural practices by preventing milk losses, which not only reduces food waste but also promotes resource efficiency. This aligns with global efforts to create more resilient and environmentally friendly food systems. Moreover, this approach can empower small-scale farmers and local mabisi producers, who are mostly women, by offering them a reliable market for their dairy products, thereby strengthening local economies and contributing to rural development. In the long run, upscaling traditional products like mabisi could enhance the sustainability of the entire food system by promoting biodiversity, and fostering resilience in the face of climate change and other challenges.

Acknowledgments

A few years ago, I started a PhD program. The journey began in 2020, a year marked by the unprecedented events of the COVID-19 pandemic. It wasn't an easy path, full of bumps, hurdles, and challenges. But four years later, here I am, I made it. Along the way, I experienced immense personal growth, learned countless lessons, discovered myself, and gained some valuable insights about other cultures. I also had the pleasure of meeting incredible people from around the globe. Your support made this possible, and I'm deeply grateful.

Special thanks to my promotor and supervisors, **Prof. Dr. Eddy Smid, Dr. Anita Linnemann, Dr. Sijmen Schoustra,** and **Dr. Bernard Moonga.** From day one, you stood by my side, instilling the confidence I needed to run this race. Even when I stumbled and fell, you remained unwavering in your support, guiding me with your wisdom and knowledge to the finish line. I'm forever indebted to you all for your invaluable scientific advise, and constructive feedback, and all the discussions that shaped the direction of this research and profoundly impacted me personally. Our meetings were always a great source of relief from stress. I'm truly fortunate to have had the opportunity to learn under your guidance and mentorship. This could not have been accomplished without your collective support. **Eddy,** you brought clarity when my mind was clouded with ideas and uncertainty, helping me find the right path forward. **Anita,** your kindness and motherly warmth encouraged me to keep moving forward whenever I hit rock bottom or my vision became blurred. You were there during countless challenging moments, bringing hope to even the most hopeless situations. **Sijmen,** you didn't just provide guidance and leadership to the project; you were the go-to person, whether in Wageningen or Lusaka. You made the sandwich programme arrangement seamless and were always accessible. The travels between Lusaka and Wageningen felt effortless because you were always on top of everything. **Bernard,** your optimistic nature never compromised your clarity. Whenever I reached out to you, you responded with something uplifting and encouraging. In moments of uncertainty and gloom, I always knew I could count on you.

I want to express my deepest gratitude to my thesis committee members. To **Prof. Inge Brouwer, Dr. Yann Madode, Dr. Irma van Rijswijck,** and **Dr. Herwig Bachmann** here is to say thank you for taking your time and review and offer feedback on my thesis.

I also express my appreciation to my co-authors for your contribution and the ultimate success of this work. **Prof. Dr. Markus Stieger,** your invaluable contribution and expertise on the sensory evaluation, data analysis and your timely responses are greatly appreciated. **Dr. John Shindano,** you provided the much needed guidance on the experimental set up of the sensory and consumer survey and for that I'm eternally grateful. **Dr. Anna Alekseeva,** your commitment, dedication, willingness to share your knowledge is unmatched. You provided your experiences and expertise in bioinformatics analysis. You were always ready to give a helping hand, in and away from the lab. I cannot thank you enough.

I also extend my heartfelt thanks to the students I was privileged to supervise during their MSc thesis, who are also my co-authors **Stefanie** and **Tomas.** Thank you for your contribution to

many experiments that form a huge part of this work. Your brilliant ideas added flavor to this thesis. I'm grateful for the growth I gained from working with you.

From the Food Microbiology group, my very first office mates in the department, **Nathalia B.** although mine was a very short stay, you warmly welcomed and gave me an orientation to Axis and the department. Thanks, **Andy** for welcoming me to the office and offering some valuable tips for a good start to the PhD. **Angela,** thanks for the stories about your experience in the Netherlands and offering a listening ear when the fear for covid 19 crept in.

And to my other office mates, **Jasper B.,** you were there to answer my questions when I was stuck with R. **Xuchuan,** besides being a good office mate, you welcomed me and me an orientation to your lab and it became home for me. **Piotr**, thanks warm morning greetings. **Johanna,** from the INREF online meetings to becoming office mates and accepting being one of my paranymphs, I'm truly thankful. **Xing,** thanks for rejuvenating me with your therapeutic tea that rejuvenated me when I was unwell. **Clara,** I knew I could always reach out to you when I needed some help, thank you so much.

To **Gerda and Marjan** (Laboratory of Genetics), thanks to both of you for your efficient administrative support throughout this journey.

Marcel Z, your leadership is both inspiring and exemplary. You've created an atmosphere that encourages everyone to thrive, making FHM a home for all. Thank you for sharing your fascinating article about freezers from nu.nl, it certainly gave me food for thought!

Denis, thanks for your kindness and for checking how things are going. I also appreciate your help in the Lab. **Yue,** I have always admired your calmness, dedication, and your sweet smile. **Judith,** thank you for facilitating the GC-MS analysis and simplifying the Chromeleon. You were always available to share your expertise and address my queries. **Oscar,** thanks for your help with interpreting the GC-MS data. **James (**and Dinah**),** thanks for your kindness and warmly welcoming me into your home for those scrumptious African meals. **Alberto G.,** my go-to person during my first interaction with R. Thank you for always being there when I reached out. **Claire,** thank you for the insightful stories and wonderful times shared during the PhD trip. **Richard,** thanks for allowing me to inconvenience you and take up your office desk. **Christina,** I loved walking with you because it always motivated me to exercise. **Soundarya,** thanks for the friendly talks and advice. **George,** you were an exceptional driver during our PhD trip in Switzerland and Germany, and I enjoyed your delicious iced coffee. **Sylviani,** thanks for the many rides I made in your car, it's always a joy to be around you; and I truly love your passion for jigsaw puzzles. **Linda,** you are such an amazing soul. You were such a good roommate in Germany during the PhD trip. **Alberto B.,** your relaxed demeanor and approachability made interactions so effortless. I'll never forget bumping into you in Germany when I was almost lost during the PhD trip. **Rebecca**, there was always a positive atmosphere in your presence; you always know how to make someone feel at home. **Tamara,** your vibrant mind is always captivating, thank you for your kindness and your constant willingness to help. **Kais,** thank you for sharing your programming insights. **Domiziana,** it was a pleasure working with you in the Lab. **Frank,** it was an awesome pleasure to know more about you during the

PhD trip. **Theo,** always enjoyed a bite of the snacks you brought to the coffee room. **Jasper Z.,** I enjoyed playing those games with you at Café Onder de Linden. **Merijn,** thanks for helping with the code. **Selena, Luuk, Zhiwei, Jodi, Gamze, Anne, Marcel T., Wilma, Martine,** and **Neda**, it was an absolute pleasure interacting with you. **Ingrid,** your stern commitment to keeping the laboratory clean and organized ensured a safe environment for all of us. Thank you for the handbook on yeasts. To **Heidy** and **Tjakko,** thank you for joining us and representing the department on the PhD trip.

I further extend my appreciation to the Laboratory of Genetics for your warmth and for accommodating me. **Bas,** your inspiring leadership and support meant a lot. **Francisca**, I greatly appreciate your invaluable help during the DNA extractions, your presence made the lab work seamless. **Corrie,** thanks for your efficiency in ensuring the availability of laboratory supplies for delivery to Lusaka. **Suzette,** I loved how well you fit into our Zambian community, and thanks for being reliable. **Ben, Eric, Michiel,** and **Gabriella,** thank you that you were always available for consultations. **Bwalya,** my associate at NISIR, it was nice to work with you away from home.

To my **INREF** family**,** thank you for the incredible support during the challenges of the COVID-19 pandemic and beyond. It kept me sane figuring out my new journey with the unexpected interruption. The teamwork and encouragement helped me survive those difficult times. I appreciate your resilience and positivity, it made an otherwise overwhelming period bearable. Thanks to all the group promotors and supervisors, **Wilfred, Gerlide, Valentia, Elise, Grace, Ruth, Peter, Agripina,** and **Juliet**. And cheers to my fellow PhD candidates: **Alanna,** they say sharing is caring and you were always ready to share any helpful insights, tips, and even household goods with the group. **Carole,** thanks for stepping in and picking up my parcels, I'm forever grateful for the insightful discussion we had with you and **Betty** (RIEP) at the start of this journey. **Blandine,** your determination has always been an inspiration and encouragement. **Sakile**, thanks for being an amazing sister's keeper and caring for me during the moments I was down. Your wise counsel has been invaluable, and I'm deeply grateful for it. **Ardinesh,** thanks to many conversations during the social lunch, I guess the chicken has finally managed to cross the road. **Chris,** thanks for always being available to help, and grateful that you gave me some hope when my paper was rejected. To my Zambian colleagues, you guys were a great company. The many dinners and fun times we shared brought balance to the journey. **Taonga,** thank you for being my amazing shopping companion during our mall-hopping adventures. You have such a great vibe and made every trip memorable. Thanks for keeping me sane when things were not making sense. Most of the time you were not even aware that you were lifting a drowning soul. **Mundia,** your positive energy is infectious, and I appreciate it. **Murambiwa, Robert, Mubonda,** and **Shepherd,** what an awesome bunch! From travels to shared meals, never a dull moment, being around you needed a certain level of grace muli bakulekafye. Bo ndate from being undergrad coursemates to tackling the PhD together, thank you for sharing our struggles and the words of encouragement that saw us conquer. C**hindi**, I'm truly grateful for always helping advertise my moving-out sales. **Bertha, Nalucha, Brian,** and **Siatwiinda** we created unforgettable memories to be cherished forever.

I would also like to express my sincere gratitude to the University of Zambia (UNZA), Department of Food Science and Nutrition members of staff and students for creating a conducive environment for my experiments and always being there to assist. Special thanks to **Dr. Hikeezi, Dr. Kumwenda, Mr. Nachibanga, Mr. Banda, Mrs Chipeta, Lister,** and **Mr. Zulu**.

I'm forever grateful to my employer, the National Institute for Scientific and Industrial Research (NISIR) for allowing me time to pursue my dream. I'm sincerely thankful to all my colleagues, **Dr. Nyambe, Mrs. Munthali, Dillon, Thomas** (IT), and **Doreen,** and the interns; **Deborah** and **Kaku** for the support rendered.

To the **Navigators International Wageningen**, you were a great blessing and I'm glad I met you. Thanks, **Steve** and **Chelsey** for inviting me to the group, **Bart** and **Jedi** for your hospitality, and **Stefanie** for leading the studies.

MaryBridget and **Mark**, thank you for your hospitality. **Chishimba** (and **Kamiel)**, my childhood friend, reconnecting with you after two decades was an absolute joy. Thank you for your support and friendship.

Esther and **Paul,** yours is my home away from mine, thank you for always having my back. This is as much your achievement as it is mine. We carried this burden together, especially in the last few weeks. I can never thank you enough.

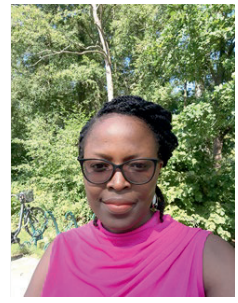
Benjamin, thanks for making the last stretch of this journey enjoyable and adventurous. You were heavenly sent.

To my late **mother** and sister, (Rodah), thank you for making me believe in myself and the power of education. Your wisdom has been my guide and strength. I see your smiling faces and know this would be a proud moment for you. To my brothers **Charles** and **Lwito**, my sister **Kasompe,** sisters-in-law **Bana Dalitso** and **Bana Moses, uncle Sydney**, **aunt Leticia**, my nieces, and nephews, thank you for your unwavering support and for always cheering me on. And to my dad, your constant encouragement meant everything.

Through it all, **Mwe Lesa** you were the peace that stilled the waters of my soul and kept me grounded. None of this would have been possible without you.

Lastly, if I missed acknowledging you, it was not intentional, and I sincerely apologize. Your support is just as appreciated as those mentioned above!

About the Author



Thelma Wakawezi Sikombe was born in Mufulira, Zambia on 05 June, 1981. After completing high school at Hillcrest Technical Secondary School, she earned a Bachelor of Science in Food Science and Technology from the University of Zambia in 2006. She then joined the National Institute for Scientific and Industrial (NISIR) as an intern and was later employed as a scientific officer at the Food Science Research Center. In 2012 she headed the Center, before proceeding for her postgraduate studies in 2014. She obtained a Master's degree in Food Technology and Nutrition from Lund University, Sweden in 2016 and returned home to continue working at NISIR.

During her master's degree studies, she studied the nutritional and functional characteristics of the Baobab fruit, where she assessed the effects of geographic conditions on its properties and explored the potential for functional food development. During the internship part of her master's program, she participated in a study evaluating the effects of breakfast meals varying in protein source on appetite regulation in healthy male adults. Her role was to examine the relationship between postprandial amino acid kinetics of the different protein test meals with the satiety responses of the subjects. This experience exposed her to different research methodology techniques. At NISIR, her research focuses on the functional characteristics of nutritious, health-promoting foods, product development, post-harvest management, and the safety of foods of indigenous nature. She also provides scientific advice and consultancy in food processing, quality assurance, food safety, and regulatory compliance. Additionally, she serves on several technical committees, including the National Standards Bureau and the National Codex Committee.

Thelma followed her dream of pursuing a PhD and in 2020 she started her program at the Laboratory Food Microbiology and Laboratory of Genetics (WUR). The program was supported by the Wageningen University Interdisciplinary Research and Education Fund (INREF) under the project, *Traditional fermented foods to promote food and nutrition security in Africa; entrepreneurship, value chains, product development and microbial ecology in Zambia, Zimbabwe, and Benin*'. Her research findings are presented in this thesis.

Publication list

Full papers

- **Sikombe, T. W**., Moonga, H. B., Schoustra, S. E., Shindano, J., Stieger, M., Smid, E. J., & Linnemann, A. R. (2023). Sensory characteristics and consumer acceptability of four variants of mabisi, a traditionally fermented Zambian dairy product. LWT, 188, 115410. https://doi.org/10.1016/j.lwt.2023.115410

- Mugode, L., Ha, B., Kaunda, A., **Sikombe, T**., Phiri, S., Mutale, R., Davis, C., Tanumihardjo, S., & De Moura, F. F. (2014). Carotenoid retention of biofortified provitamin A maize (Zea mays L.) after Zambian traditional methods of milling, cooking and storage. Journal of Agricultural and Food Chemistry, 62(27), 6317-6325.

- **Sikombe, T. W.,** Linnemann, A. R., Moonga, H. B., Quilitz, S., Schoustra, S.E., Smid, E. J., and Alekseeva. A. *(year)*. Odour-active aroma compounds in traditional fermented dairy products: The archetypical case of mabisi that supports food and nutrition security in Zambia. *(submitted).*

- **Sikombe, T. W.,** Moonga, H. B., Linnemann, A. R., Smid, E. J., Schoustra, S. E. *(year)*. The effect of storage temperature on the functional characteristics and microbial community of traditionally fermented dairy products. *(submitted).*

Conferences presentations

- **Sikombe, T. W**., Moonga, H. B., Schoustra, S. E., Shindano, J., Stieger, M., Smid, E. J., & Linnemann, A. R. Sensory characteristics and consumer acceptance of four different types of traditional Mabisi. Paper presented at 3rd Agricultural Symposium from 29 - 30 September 2022, University of Zambia, Lusaka, Zambia.

- **Sikombe, T. W**., Alekseeva, A., Quilitz, S., Moonga, H. B., Schoustra, S., Smid, E., & Linnemann, A. (2023). The microbial community of a traditional Zambian dairy product generates different aroma profiles upon variation of production methods. Poster presented at the 14th International Symposium on Lactic Acid Bacteria, 27 - 31 August, 2023, Egmond aan Zee, Netherlands.

- **Sikombe, T. W**., Alekseeva, A., Quilitz, S., Moonga, H. B., Schoustra, S., Smid, E., & Linnemann, A. Aroma-active compounds of four variants of Mabisi, a traditionally fermented Zambia dairy product. Poster present at the 37th EFFoST International Conference, 6 - 8 November, Valencia, Spain.

Overview of completed training activities

Name of the course / meeting	Organizing Institute (s)	City	Year
Category A: Discipline specific activities			
Healthy food design	VLAG	Online	2021
Healthy and sustainable diets: synergies and trade-offs	VLAG	Online	2021
Reaction kinetics in food science	VLAG	Online	2020
International Advanced Course on Sensory perception & food preference: Into the future!	VLAG	Online	2020
16S rRNA workshop 1	ZIEL	Online	2021
16S rRNA workshop 2	ZIEL	Freising (DE)	2022
Chemometrics	VLAG	Wageningen (NL)	2022
7th Africa Higher Education Week and RUFORUM Triennial Conference	RUFORUM	Online	2021
3rd Agricultural Symposium at University of Zambia	UNZA	Lusaka (ZM)	2022
Protein quality, evaluation and application course	VLAG	Wageningen (NL)	2023
14th International Symposium on Lactic Acid Bacteria (LAB 14)	LAB committee	Egmond aan Zee (NL)	2023
37th EFFoST International Conference	EFFoST	Valencia (ES)	2023
NLSEB 2024 Conference	NLSEB	Ede (NL)	2024
General courses			
Reviewing a Scientific Manuscript	WGS	Wageningen (NL)	2020
Project and time management	WGS	Online	2020
Brain-friendly working and writing	WGS	Online	2020
Research data management	WGS	Online	2020
Scientific integrity	WGS	Online	2020
Searching and organizing literature	WGS	Online	2020
Introduction to R	VLAG	Online	2021
PhD Carousel	WGS	Online	2021
Ethics and philosophy in food science and technology	VLAG	Wageningen (NL)	2022
Applied statistics	VLAG	Wageningen (NL)	2022
PhD Mid-term Retreat	PE & RC	Ede	2022
Scientific writing	WGS	Online	2023
Last stretch of the PhD and proposition writing	WGS	Wageningen (NL)	2023
Effective and efficient communication in academia and beyond	WGS	Wageningen (NL)	2023
Effective behavior in the professional surrounding	WGS	Online	2023
Adobe Illustrator - Scientific Artwork and Infographics	WGS	Online	2024

Other activities

Preparation of research proposal	FHM/Laboratory of Genetics	Wageningen / Lusaka	2020
Advanced fermentation course	FHM	Wageningen (NL)	2022
PhD study tour to Germany and Switzerland	FHM	DE & CH	2022
Bi-weekly group meetings, seminars, and departmental colloquia	FHM/Laboratory of Genetics	Wageningen (NL) / Online	2020 - 2024

Colophon

The research described in this thesis was performed at the Laboratory of Genetics and Laboratory of Food Microbiology, Wageningen University & Research, The Netherlands, and partly at the Food Microbiology and Food Chemistry Laboratory of the University of Zambia.

The research was funded by the Integrated Research Fund (INREF) of Wageningen University under the project entitled *Traditional fermented foods to promote food and nutrition security in Africa.*

Financial support from Wageningen University for printing the thesis is gratefully acknowledged.

Cover design by: Cloé Villard and Thelma Sikombe

Thesis layout by: Thelma Sikombe

Printed by: ProefschriftMaken