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Influence of fermentation temperature on microbial community composition and physicochemical properties of mabisi, a traditionally fermented milk

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ABSTRACT

Fermentation temperature is a crucial factor in the production of fermented dairy products. This study investigated the influence of fermentation temperature on physiochemical properties and the composition of the microbial communities of two types of mabisi: tonga and barotse mabisi. Mabisi is a traditionally fermented milk product made in Zambia by spontaneous fermentation of raw milk at ambient temperature. The two types of mabisi were produced with tonga mabisi fermented at 20, 22, 25 and 30 °C, and barotse mabisi at 25 and 30 °C. The pH, titratable acidity, syneresis, consistency, microbial community composition and aroma compounds were determined. We found that fermentation at 20 and 22 °C was slower than at 25 °C but they all produced tonga mabisi with medium acidity (pH 4.2) and 'medium' consistency within 48 h. Fermentation was most rapid at 30 °C but resulted in a product with a 'thin' consistency. Fermentation temperature did not affect the microbial community composition of tonga mabisi, which remained diverse and complex with *Lactococcus* as the dominant fermenting genus. However, in barotse mabisi, at 25 °C, the product had 'thick' consistency and the microbial community composition was dominated by *Lactococcus* whereas at 30 °C the product was thicker, more acidic and dominated by *Lactobacillus*.

1. Introduction

Dairy fermentations require the activity of microbes. Many of the commercially produced fermented dairy products use specific starter cultures, which have been carefully selected to deliver desirable organoleptic and quality attributes to the product. The most common microbes used in dairy fermentations belong to the group of lactic acid bacteria (LAB) but yeast and moulds can also be used for certain products such as kefir and surface ripened cheese, respectively (Garofalo et al., 2015; Gripon, 1993; McSweeney, 2004). The primary role of LAB is the production of lactic acid which reduces the pH of milk to values below the iso-electric point of caseins (i.e. pH 4.6) and thereby induces coagulation of the milk (Beresford et al., 2001). The different species of lactic acid bacteria grow at different temperatures.

To ensure that only the desirable bacteria grow and impart the desired quality attributes on the specific product, the optimum fermentation conditions should be put in place. Amongst these, temperature is critical because using wrong temperature during fermentation may promote the growth of undesirable and harmful bacteria, which can cause spoilage and foodborne infections. A good example of a fermentation process where temperature is strictly controlled, is yoghurt production. The starter culture is inoculated into heat treated milk at the moment when the milk has been cooled to 45 °C, which is the optimum temperature for starter growth. The species used are *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* which, are both classified as thermophilic lactic acid bacteria (LAB) suited to grow at relatively high temperature (Lee & Lucey, 2010). These microbes will coagulate the milk to the desired consistency/texture and deliver the characteristic aroma and flavour. For cheese production, usually mesophilic LAB such as *Lactococcus lactis* and *Leuconostoc mesenteroides* are

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used, which thrive at slightly lower temperatures (20–39 °C) (Johnson, 2013) compared to the thermophilic LAB. These examples show the importance of establishing the appropriate process conditions for the production of a fermented dairy product. This type of information is especially important for the development and optimisation of artisanal or traditional dairy fermented products such as mabisi.

Mabisi is a traditionally fermented milk product from Zambia, which is made by spontaneous fermentation of raw bovine milk in a calabash or gourd at ambient temperature for up to 48 h after which, it is stirred and ready for consumption (Moonga et al., 2019; Schoustra et al., 2013). No defined starter culture is used in this traditional fermentation. Previous work has shown that the microbial community is dominated by around six to up to eight different species of lactic acid bacteria (Schoustra et al., 2013) which, include both mesophilic and thermophilic bacteria. Mabisi is a popular and versatile product with several uses in the diet as it can be consumed with the main meal, as a dessert, snack or a beverage by both children and adults (both women and men). Its consumption is wide spread throughout the country but the production is not formally regulated due to lack of information on product characteristics and optimum production process conditions. A recent study on mabisi identified seven different production methods including tonga and barotse mabisi (Moonga et al., 2019). Tonga mabisi involves a spontaneous fermentation of raw milk in container without the removal of whey whereas barotse mabisi in addition, involves several steps of alternate removal of whey and addition of raw milk. Some of the critical processing parameters include fermentation temperature and duration, which have been associated with increased whey content in the product (watery mabisi) by consumers (Moonga, 2019). This process of whey separation from the curd is referred to as syneresis, which can influence the rheological properties of mabisi.

The importance of incubation temperature is highlighted by the producers' choice of fermentation location in order to minimize temperature fluctuations. Most mabisi fermentations are carried out in a cool place in the house but production can be accelerated by placing the container outside directly in the sun especially during the cold months or slowed down by putting it in a water-bath to buffer the effect of high temperatures during the hotter months. Producers also reported that fermentation takes longer in a new container than an old one that was repeatedly used for mabisi production (Moonga et al., 2019). With the establishment of more milk collection centres (MCC) and promotion of value addition within value chains of local products in the country, there is need to establish standard processing conditions and document product characteristics for mabisi.

The ambient temperatures in Zambia vary from one season to another, leading to the following division, each with a specific temperature range during the day/night: Hot dry (Sept–Nov.), 18–35 °C; hot wet (Dec.–April), 16–32 °C and cold (May–July), 8–28 °C (Mulenga et al., 2016). This implies that fermentation temperatures differ throughout the year and given the fact that the microbial communities responsible for fermentation of mabisi consists of both mesophilic and thermophilic bacteria, variations in temperature could have an impact on their relative abundance. This in turn, may lead to variation in the quality of the product. Therefore, the aim of this study was three-fold: (i) the first aim was to investigate the influence of fermentation temperature on quality parameters and microbial community composition changes in mabisi by examining the production of mabisi in a single batch as well as multiple batches at different temperatures that mimic the seasonal variations and producer practices. (ii) The second aim was to assess the effect of using a new fermentation container as opposed to one previously used and (iii) the third aim was to compare two different production methods: tonga and barotse mabisi. This study provides insights on how the differences in microbial community composition as related to fermentation temperature fluctuations impact the quality of mabisi.

2. Material and methods

2.1. Mabisi preparation and experimental layout

Two methods of mabisi production were used in this study to produce tonga and barotse mabisi. Their respective production flow diagrams are shown in Fig. 1.

The study was divided into four experiments. Tonga mabisi was used to study the effect of temperature using four different fermentation temperature regimes i.e. water temperature (20 °C (WT)), room temperature (22 °C (RT)), 25 °C and 30 °C which, cover the range of seasonal temperature variations. The experimental set-up is shown in Fig. 2. Water temperature means that the fermentation container was placed in a water bath filled with tap water with an average temperature of approximately 20 °C.

2.1.1. Single batch production of Tonga mabisi

The first experiment involved the production of mabisi in a single batch of raw bovine milk split into triplicate and fermented under four different fermentation conditions with three sampling time points (Fig. 2a). The fresh raw milk was obtained from the University of Zambia (UNZA), School of Agricultural Sciences Field Station in a new clean (sterile) 5-litre plastic container. Out of this, 400 ml was then poured into each clean pre-washed 500 ml plastic bottle, closed with a lid and left to ferment for up to 72 h according to the production flow diagram (Fig. 1a). Each given temperature was allocated nine bottles, three for each time point of sampling indicated as R1, R2 & R3 (Fig. 2a), which were three biological replicates.

2.1.2. Multiple batch production of Tonga mabisi

The set up was similar to the single batch production in terms of fermentation temperature and sampling times but the difference was that three independent batches of raw milk were used to produce mabisi on 3-day intervals per batch. These repeated production batches were the biological replicates, which were all fermented in new containers (Fig. 2b).

2.1.3. Fermentation in a used container

The multiple batch production in new containers was simultaneously executed in previously used containers at the same temperature and same time series of sampling points. The 500 ml bottles previously used for mabisi fermentation were washed with hot water prior to filling with raw milk. Three batches of production were made on the same days as those in section 2.1.2 using the same raw milk for each batch as shown in the layout (Fig. 2b).

2.1.4. Barotse mabisi

The barotse mabisi was fermented at only two temperatures, 25 °C and 30 °C with four time points for sampling. The raw milk was also obtained from the UNZA Field Station from one batch with 400 ml filled in each of the new (sterile) clean 500 ml plastic bottles. The layout was similar to the single batch production (Fig. 2a) but only performed at two temperatures following steps provided in the production flow diagram (Fig. 1b). Three (3) bottles were incubated at each temperature (as replicates) for three days after which the whey was removed and fresh raw milk added up to 400 ml. They were allowed to ferment for another day (fourth day) at their respective temperature and again the whey was

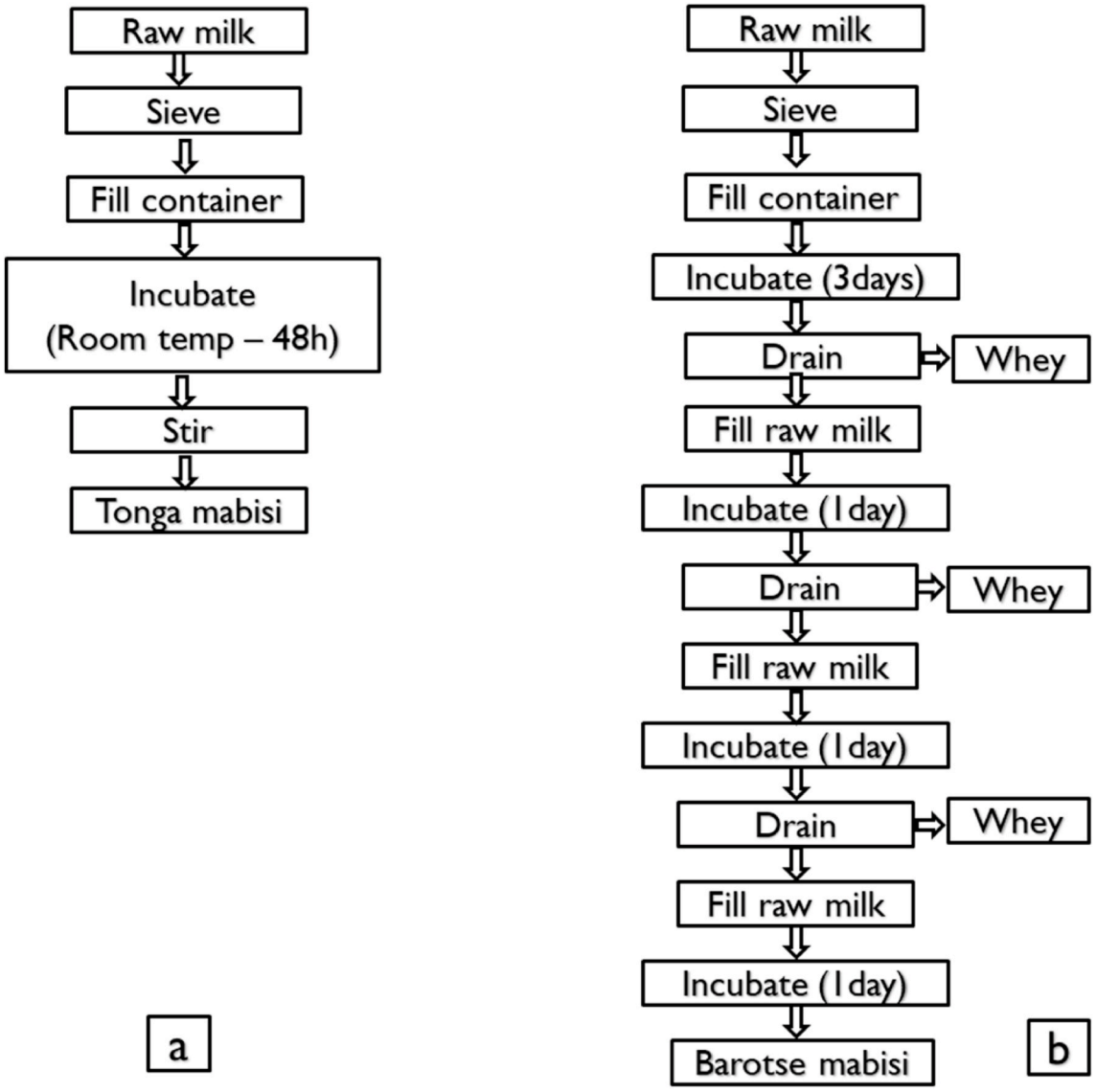


Fig. 1. Production flow diagram of tonga (a) and barotse (b) mabisi.

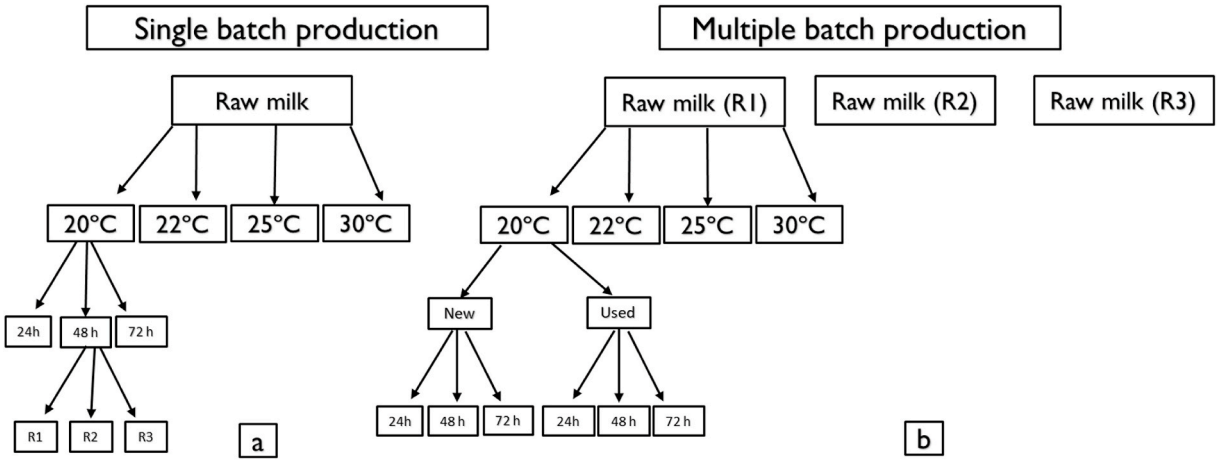


Fig. 2. Experimental layout of single batch (a) and multiple batch (b) production of tonga mabisi. R1-3 are biological replicates, taken at sampling time (24, 48 & 72 h), fermentation temperature (20°, 22°, 25° & 30 °C) and type of fermentation container (new plastic container and used plastic container (previously used for mabisi production)).

removed followed by addition of raw milk. This was repeated for one more day (fifth day) and the final product was obtained on the sixth day. The samples of whey removed and final product were retained for physicochemical and microbial analysis. The volume of whey removed was also measured and used to determine product yield using the following formula:

$$\text{Product Yield } (\%) = (\text{Final volume of end product} / \text{Total volume of raw milk used}) * 100$$

2.2. Physicochemical analysis

The mabisi samples were analysed for pH, titratable acidity (TTA), syneresis and consistency. pH was analysed by a digital pH meter (Hanna HI 8424) and the acidity was analysed according to the AOAC official methods (AOAC., 2005). Syneresis was analysed by measuring the volume of whey separated from the curd and calculating its percentage of the initial volume of raw milk. Consistency was analysed using the Adam's consistometer, which measures the diameter of spread of a semi-liquid product after 30s (Garcia & Wayne, 1998; Gould, 1992).

2.3. Microbial community composition

The mabisi samples were analysed by a culture independent method. The DNA was extracted and purified as described by Schoustra et al. (2013) by carrying out the following steps, first 1 mL of fermented milk was spun down (2 min, 12,000 RPM), after which the supernatant was removed. The cells were re-suspended in a mix of 64 μL EDTA (0.5 M), 160 μL Nucleic Lysis Solution, 5 μL RNAse, 120 μL lysozyme and 40 μL pronase E. After an incubation time of 60 min at 37 °C and agitation of 350 RPM, 400 μL ice-cold ammonium acetate (5 M) was added and the mixture was cooled on ice for 15 min. The mixture was spun down and 750 μL of 51 supernatant was transferred to a tube containing 750 μL phenol. This tube was vortexed and its content spun down (2 min, 12, 000 RPM) and 500 μL of supernatant was transferred to a tube containing 500 μL chloroform. This tube was vortexed and its content spun down (2 min, 12,000 RPM) and 400 μL of supernatant was transferred to a tube containing 1 ml 100% ethanol and 40 μL sodium acetate (3 M). This DNA containing tube was left to precipitate at −20 °C overnight. The next day, the tube was spun for 20 min at 12 000 RPM at 4 °C. The supernatant was carefully aspirated, and the DNA pellet was washed by adding 1 mL 70% ethanol. The tube was spun for 10 min at 12 000 RPM at 4 °C, after which the supernatant was aspirated again. The DNA pellet was left to dry at room temperature and dissolved in 20 μL 10 mM Tris pH 7.5. The extracted DNA was sent for bacterial 16 S rRNA gene amplicon paired-end sequencing of the V4 hypervariable region (341 F-785 R) on the MiSeq Illumina platform performed by LGC genomics (Berlin, Germany). Since previous work showed that yeast is only sporadically present in mabisi (Schoustra et al., 2013), we focussed on the bacterial community composition in the present work.

For further data processing and statistics, the QIIME pipeline (Caporaso et al., 2010), modified by (Bik et al., 2016) was used. Paired-end reads were joined using join_paired_ends.py (with minimum overlap 10 basepairs) after which sequences were trimmed and filtered using cutadapt (v1.11 -q 20, -m 400 (Martin, 2011)) using the known primer sequences CCTACGGGNGGCWGCAG and GACTACHVGGGTATCTAAKCC to trim both sides of the sequence. These trimmed sequences were then checked for chimera's, using uchime (v4.2.20, gold database (Edgar et al., 2014)), with sequences of a lower chimera score than 0.28 retained. After trimming, filtering and a quality check using pick_open_reference_otus.py (-s 0.1, -enable_rev_strand_match TRUE, -align_seqs_min_length 75, -pick_OTU_similatiry 0.95), the sequences were clustered into operational taxonomic units (OTUs). Taxonomy of the resulting OTUs was assigned to representative sequences using the Greengenes (v13.5) rRNA database. This algorithm gives a representative sequence for an OTU, which is subsequently, used to perform a local blast using the gold database from uchime. The taxonomy from the top ranking BLAST hit was used for further data processing.

2.4. Aroma compounds

Volatile organic compounds (VOC's) in the mabisi samples were measured using Headspace-Solid Phase Microextraction Gas Chromatography-Mass Spectrometry (HS-SPME GC-MS) with a Trace 1300 Gas Chromatograph (Thermo Fisher) coupled to a TriPlus RSH autosampler (Thermo Fisher) and an ISQ QD mass spectrometer (Thermo Fisher). Frozen samples were incubated at 60 °C for 20 min. Volatile compounds were extracted for 20 min at 60 °C using an SPME fiber (Car/DVB/PDMS, Supelco). The compounds were desorbed from the fiber for 2 min onto a Stabilwax®-DA column (30 m length, 0.25 mm ID, 0.5 μm df, Restek). The PTV was heated to 250 °C and operated in split mode at a ratio of 1:25. The GC oven temperature was kept at 40 °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 were collected over a range of m/z 33–250 in full-scan mode with 3.0030 scans/second. Data were analysed using Chromeleon® 7.2. The ICIS algorithm was used for peak integration and the NIST main library to match the mass spectral profiles with the profiles of NIST. Peak areas were calculated using the MS quantification peak (highest m/z peak per compound). The peak area data were normalised and used construction heat maps using multiple experiment viewer (Mev) version 4.9.0 software. We carried out a hierarchical clustering on all mabisi sample data.

2.5. Statistical analysis

The data was analysed using analysis of variance (ANOVA) at 95% significance level and mean comparisons were performed by Turkey test at 95% significance level using Statistical Package for the Social Sciences (IBM SPSS) version 22 software.

3. Results and discussion

The main objective of this study was to determine the influence of fermentation temperature on microbial community composition and quality of mabisi by using single or multiple production, new or used containers and by comparing two production methods. The results are presented in four sections representing four different (but complementary) experiments.

3.1. Effect of temperature on single batch mabisi production

3.1.1. Physicochemical properties

We have studied the changes in the physicochemical properties of mabisi fermented at four different temperatures determined in triplicate from a single batch milk production using new plastic containers (Fig. 3).

Mabisi is made from raw milk, which has a pH ranging from 6.6 to 6.8. The pH and titratable acidity (TTA) changes during fermentation of tonga mabisi are shown in Fig. 3. Only samples fermented at 30 °C

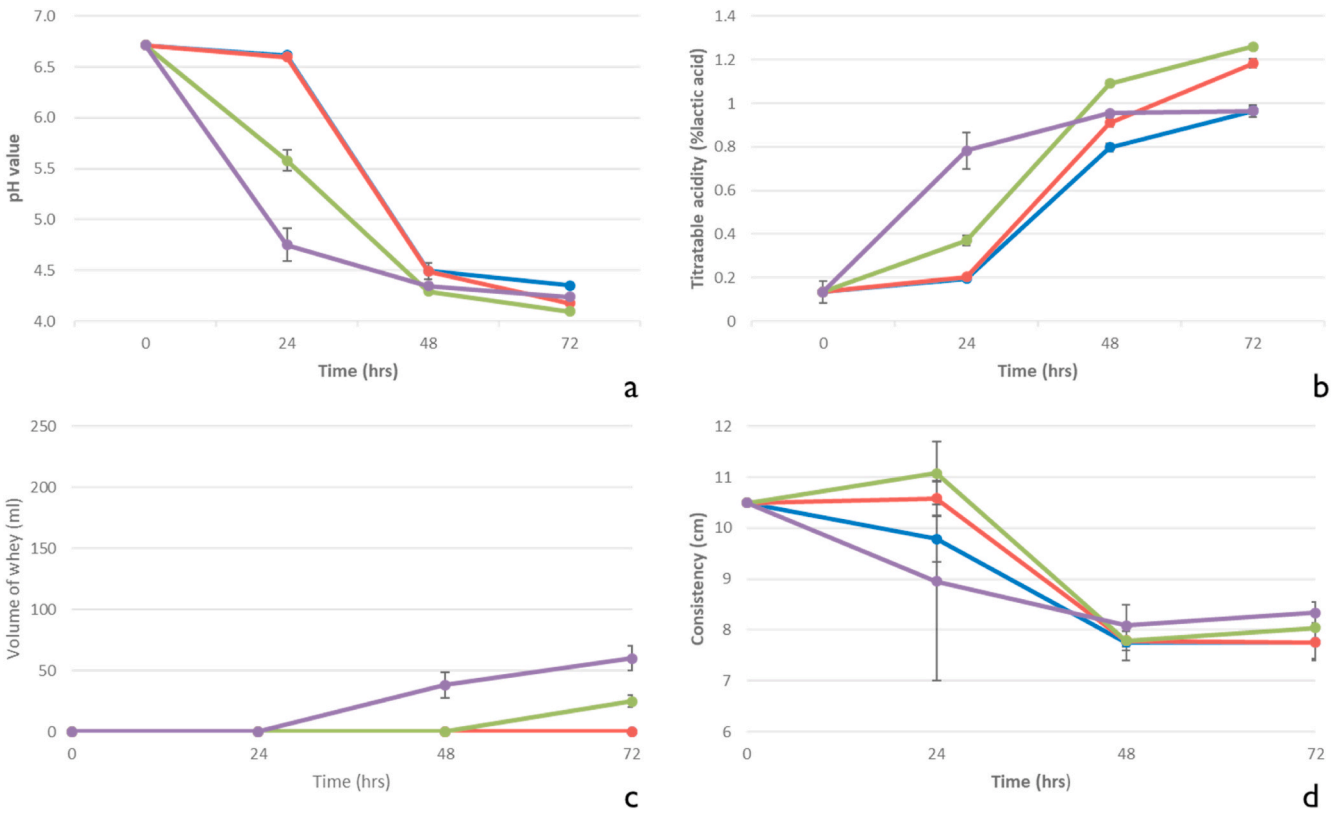


Fig. 3. Effect of temperature on physicochemical properties of tonga mabisi during a single batch production. The figure shows changes of: panel a. pH, panel b. TTA, panel c. degree of syneresis and panel d. consistency with fermentation time in hours. The colour blue denotes the temperature 20 °C, red for 22 °C, green for 25 °C and purple for 30 °C. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

coagulated after 24 h reaching pH 4.6–4.8 while those incubated at 25 °C coagulated after 36 h and those at 20 °C (water temperature (WT)) and 22 °C (room temperature (RT)) both showed coagulation after 48 h of incubation reaching pH 4.5. After 72 h, the lowest pH of 4.1 was attained by samples incubated at 25 °C and the highest (pH 4.4) by samples incubated at 20 °C. There were no significant differences in pH of mabisi incubated at different temperatures after 48 and 72 h ($p > 0.05$, Table A1).

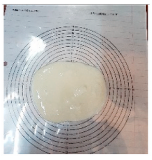
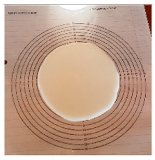
This range of pH values obtained after 48 h confirms what has previously been reported for mabisi by Schoustra et al. (2013). Moreover, similar pH ranges were reported for other traditional fermented dairy products from across Africa such as *nunu* (Akabanda, 2010), *amasi* (Feresu & Nyati, 1990; Gran, Wetlesen, et al., 2003; Mutukumira, 1995) and *kivuguto* (Karenzi et al., 2013).

Syneresis is the separation of whey from the curd after coagulation and this can affect the quality of mabisi especially in terms of consistency and viscosity. Syneresis is desirable in products such as cheese where whey is a by-product but not in products like yoghurt and mabisi. No syneresis was observed in the single batch production of tonga mabisi after 24 h for each of the different temperatures (Fig. 3c). However, the whey separation started soon after and reached 10% after 48 h for the samples incubated at 30 °C. This continued to increase until 15% at 72 h. For samples incubated at 25 °C, the whey separation started after 48 h and reached a final value of 6% at 72 h. Little or no syneresis was observed for samples incubated at 20 °C and 22 °C (Fig S1). This shows that a higher incubation temperature correlates with a higher degree of syneresis. A similar correlation has been reported for cheese making during curd processing (Walstra, 1993). Syneresis in the range between 14 and 19% has been reported in a Namibian traditionally fermented buttermilk, *Omashikwa* (Bille et al., 2007) but it should be pointed out that such observations have not been reported often for many traditionally fermented milk products found in Africa.

Next, the effect of fermentation temperature on consistency (thickness) of mabisi was determined using the Adam's consistometer. For this a classification system with 4 classes was developed (Table 1) based on the observation of diameter of spread of the product in centimetre (cm).

Mabisi production begins with raw milk that has a very thin consistency of over 10.5 cm in diameter of spread. This diameter reduces

Table 1
Classification of mabisi consistency.

Class	1	2	3	4
Consistency	Very thick	Thick	Medium	Thin
Diameter of spread (cm)	<6	6–7	7.1–8	8.1–9
Visual				

over the course of fermentation bringing about "medium" to "thick" (6–8 cm) consistency once the milk has coagulated and this can be observed between 24 and 48 h of fermentation (Fig. 3d). This reduction in diameter of spread is essentially an increase in consistency from thin to thick, which can also be expressed in terms of classes 1–4 as indicated in Table 1. The fermentation temperature, which shows a change in consistency at 24 h is 30 °C, where a "thin" or class 4 consistency (9 cm) was attained for samples fermented at this temperature. These samples went on to reach their peak consistency of nearly "medium" or class 3 (8.1 cm) at 48 h but thereafter, became thin again as a result of the high degree of syneresis (15%) after 72 h (8.3 cm). The consistency of the products fermented at 25 °C, 20 °C and 22 °C all reached class 3 (7.8–7.9 cm) at 48 h with those fermented at 20 °C and 22 °C retaining this consistency at 72 h but the one at 25 °C became slightly thinner at 72 h (8.0 cm) due to syneresis (Fig. A1).

Few studies have analysed the consistency of traditionally fermented milk products. A study on *amasi* from Zimbabwe by Mutukumira (1995) only assessed consistency using a sensory panel to score samples on a 5-point hedonic scale, which was also done by Bille et al. (2007) on *omashikwa* of Namibia though they went further to analyse the viscosity of the fermented product. Other studies analysed the viscosity of commercial sour milk (*amasi* variants) from South Africa (Moyane & Jideani, 2013) and the Rwandan *kivuguto* variants during starter culture development (Karenzi et al., 2015) but there is no classification in place for such products. Hence, this study proposes the use of this classification system for such products in future studies or for product differentiation as is the case for stirred and set yoghurt (Lucey, 2004).

3.1.2. Microbial community composition and aroma compounds

A diverse composition of the bacterial communities was found in all mabisi samples regardless of the fermentation temperature applied (i.e. 25 °C and 30 °C) (Fig. 4a). The most dominant genus responsible for the fermentation was found to be *Lactococcus*, which was present in all the samples analysed with relative abundances ranging from 13% to 42%. The other major genera present include *Acinetobacter, Aeromonas, Citrobacter, Enterobacter, Escherichia, Klebsiella,* and *Serratia*. The same genera were reported by Schoustra et al. (2013) in an earlier study on mabisi except for *Aeromonas, Enterobacter, Escherichia* and *Serratia*. The latter four genera were however found in a South African fermented milk, *amasi* (Osvik, 2013). Schoustra et al. (2013) also reported the presence of *Lactobacillus, Streptococcus* and *Leuconostoc* but these genera were present in very low relative abundances (<1%) in the tonga mabisi studied here. A recent study by Moonga et al., 2020, revealed that the bacterial composition of mabisi depended on geographical location, production method and pH of the product.

The volatile compounds detected in tonga mabisi were analysed and the results are shown in a heat map (Fig. 4b). A complex mixture of 15 esters, 7 ketones, 6 aldehydes, 3 alcohols, 2 terpenes and 6 fatty acids was found in the collective fermented product compared to mainly fatty acids in raw milk. Individual compounds are fully listed in Table A2. Temperature does not have a clear differentiating effect on the aroma profile of tonga mabisi produced in a single batch production. This could be explained by the fact that different microbes could perform similar metabolic functions within the microbial community. Thus, while we observed shifts in microbial community composition, the metabolic profiles we measured, which are the output of the combined metabolism of all microbes present have remained rather similar. The result found here is in line with previous work, that showed similar volatiles compounds profiles. Slight shifts in the volatile compounds produced by microbial communities in tonga mabisi were only observed in the 10th cycle (batch) of production in another study (Moonga, 2019). In other similar traditionally fermented products like *amasi*, 4 alcohols, 4 aldehyde, 4 ketones and 2 esters were reported (Gadaga et al., 2007; Gran, Gadaga, et al., 2003) while fermented buttermilk from Ethiopia reported 5 alcohols, 4 aldehydes, 5 ketones and 1 ester (Gebreselassie et al., 2016) and *kivuguto* variants reported fewer compounds (Karenzi et al., 2015).

3.2. Effect of temperature on multiple batch mabisi production

3.2.1. Physicochemical properties

To assess the degree of variation from one batch to another, three different batches of milk were used to produce mabisi on different days

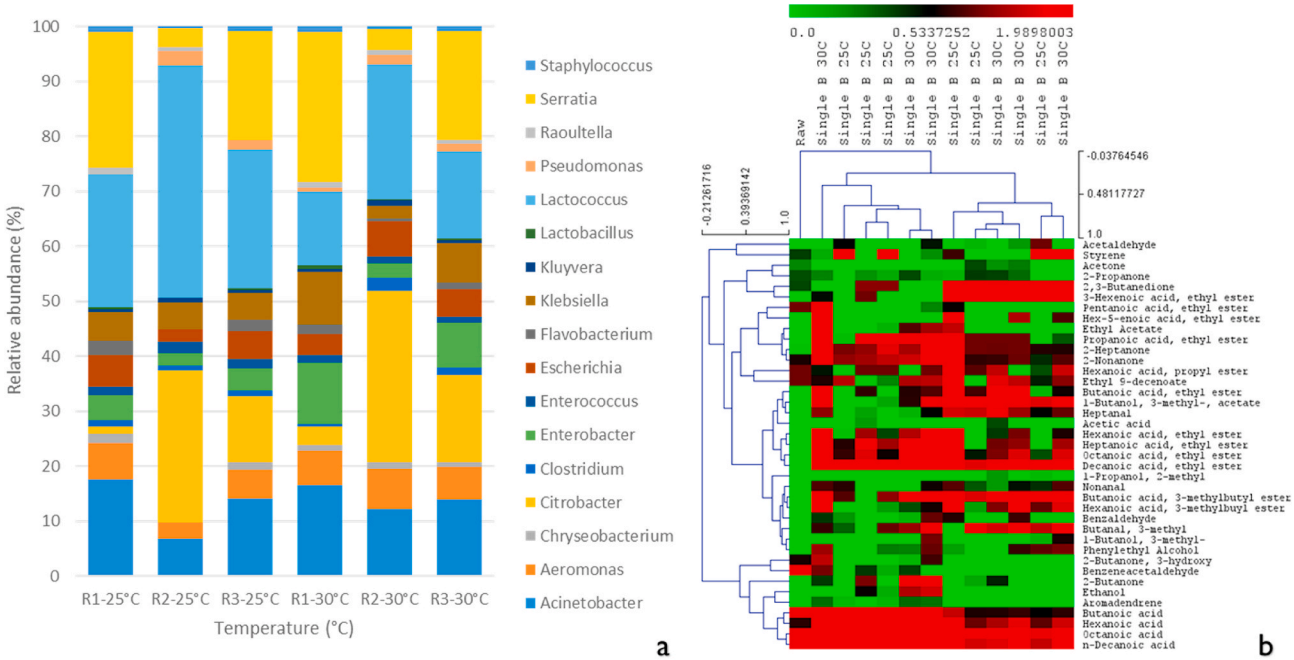


Fig. 4. Microbial community composition and aroma compounds of tonga mabisi produced from a single batch production. The figure shows: panel a. the relative abundance (%) of the top 20 microbes at genus level (y-axis) in mabisi fermented at two temperatures, 25° & 30 °C (x-axis) with R1-3 as replicates and panel b. a heat map of volatile compounds from the same product with the colour green to red showing low to high levels of volatile compounds. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

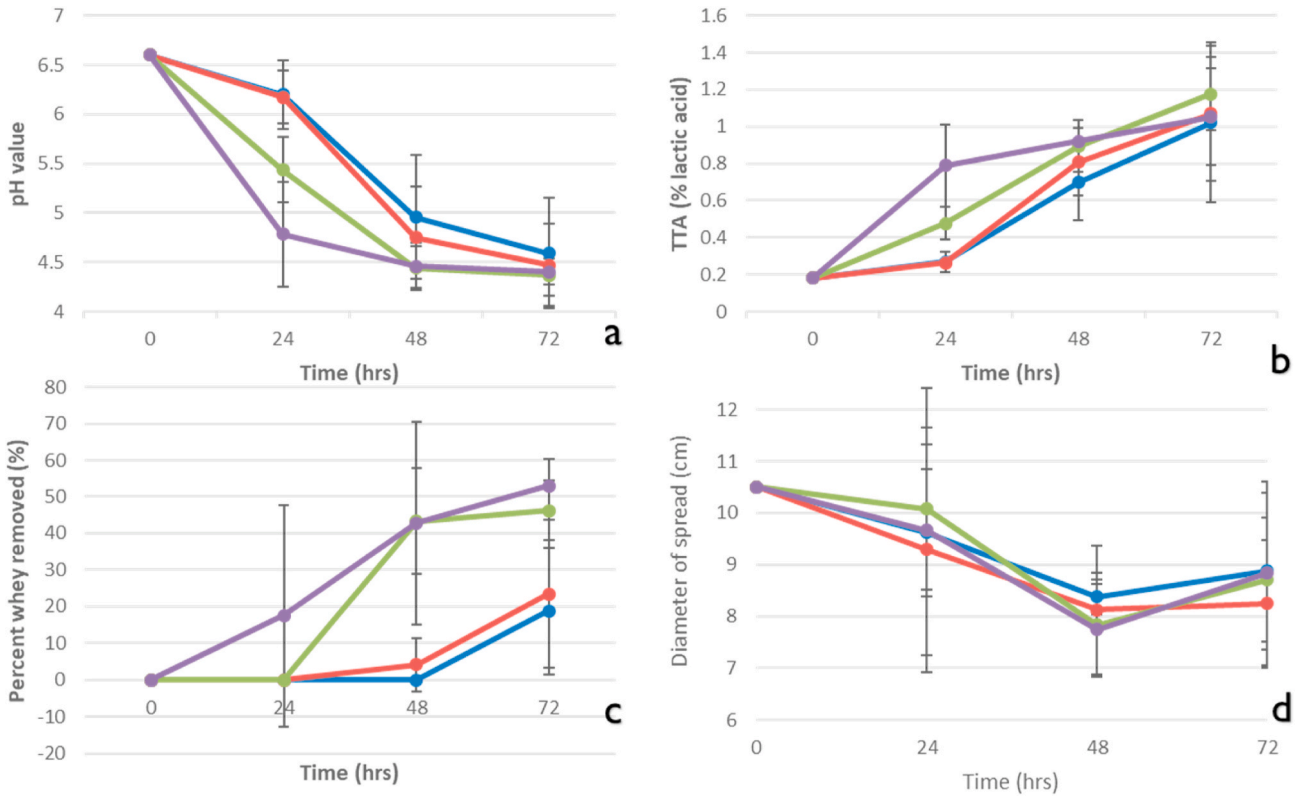


Fig. 5. Effect of temperature on physicochemical properties of tonga mabisi produced from multiple batch production. The figure shows changes in: panel a. pH, panel b. TTA, panel c. degree of syneresis and panel d. consistency with fermentation time (hours). The colour blue denotes the temperature 20 °C, red for 22 °C, green for 25 °C and purple for 30 °C. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

as biological replicates of tonga mabisi fermented at four different temperatures using new plastic containers. The pH values found (Fig. 5a) were similar to those observed in the single batch production in section 3.1.1 but there was a larger reduction in pH for samples incubated at 20 °C (WT) & 22 °C (RT) after 24 h to 6.1. However, this trend slowed down at 48 h only reaching pH 4.9 and 4.7, respectively and finally, ending with pH 4.5 after 72 h. This could be the result of ambient temperature fluctuations since these fermentations were conducted on different days and could have had a slight bearing on the room and water temperatures under which they were incubated. On the other hand, the lowest pH attained after 72 h of fermentation at 25 °C and 30 °C was 4.4. This pH was higher than that observed in section 3.1 but

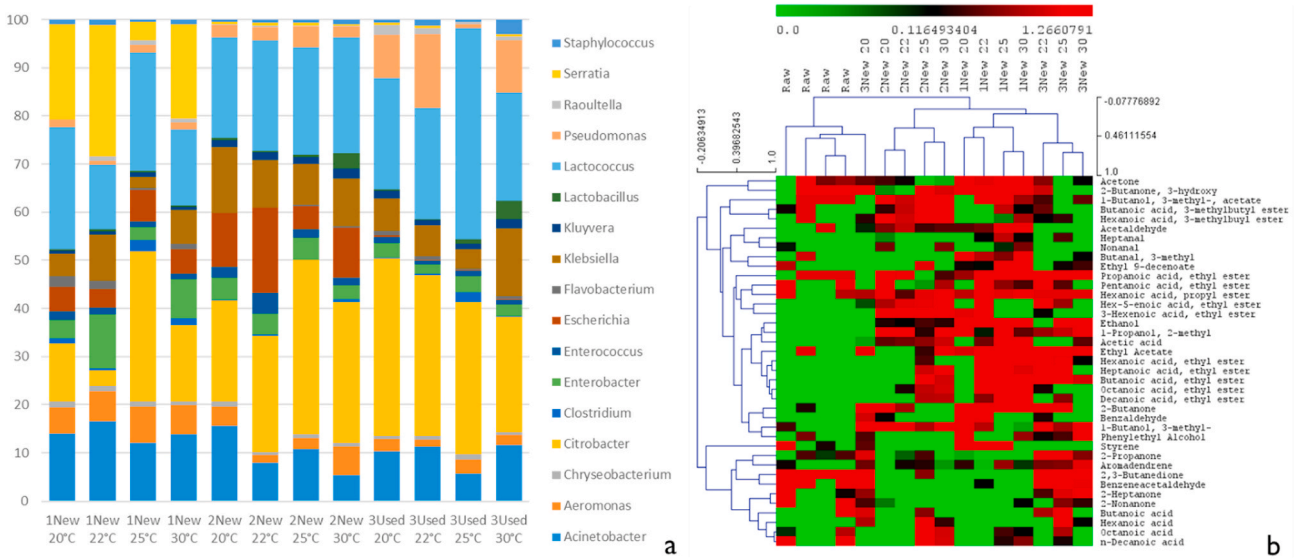


Fig. 6. Microbial community composition and aroma compounds of multiple batch mabisi production in new containers. Panel a. The relative abundance (%) of the top 20 microbes at genus level in mabisi fermented at four temperatures, 20°, 22°, 25° & 30 °C. Panel b. Heat map of volatile compounds from the same products with the colour green to red showing low to high levels of volatile compounds, respectively. Hierarchical clustering of the samples is also shown in the heat map. The batch number is indicated as 1, 2, & 3 on each bars. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the fermentation trends were similar and reproducible. The latter temperatures were not affected by ambient temperature fluctuations, since they were kept constant throughout the fermentation in respective incubators. In general, multiple batch mabisi production showed larger standard deviation than the single batch, indicating considerable variation from one batch to another probably due to differences in raw milk quality and the differences in the composition of the initial microbial community present at the onset of each fermentation.

Early onset of syneresis was observed in samples fermented at 30 °C with time point 24 h reaching 18%, which continued to increase to 43% after 48 h and ending at 53% after 72 h (Fig. 5c). For those batches of mabisi fermented at 25 °C, syneresis started after 24 h rising to 45% at 48 h then reaching a maximum of 48% after 72 h. Syneresis for the samples fermented at 20 °C only started after 48 h rising to 19% after 72 h whereas for those fermented at 22 °C, it was 5% at 48 h and then further increased to 23% after 72 h. There was generally more syneresis in this experiment at all temperatures than in the single batch production experiment (section 3.1).

The consistency (thickness) trends for samples fermented at 25 °C and 30 °C were similar to those observed in the single batch production but a slightly thicker consistency (class 3; 7.8 cm) was attained at 48 h (Fig. 5d). A lower consistency (class 4; 9 cm) was observed after 72 h due to a higher degree of syneresis. The consistencies of samples from batches fermented at 20 °C and 22 °C were also lower than those in the single batch production both at 48 h and 72 h (class 3) as they both fell in class 4.

3.2.2. Microbial community composition and aroma compounds

The multiple batch variation in the composition of the microbial communities was determined (Fig. 6a). In this experiment, results for all four temperatures are presented. The mabisi samples were also dominated by *Lactococcus* sp. (21–24%) but *Lactobacillus* sp. were also observed in samples incubated at 30 °C with a higher relative abundance (3%) than the single batch production (<1%). The other differentiating aspect was the much lower proportion of the genus *Serratia* and higher presence of *Pseudomonas*. Apart from that, the overall microbial compositions found in the two experiments were similar.

The aroma compounds were similar to those found in the single batch production even at lower temperatures of 20 °C and 22 °C which were not analysed in that experiment. The only exception is that there was a lower relative abundance of fatty acids observed in this experiment than in the single batch production (Fig. 6b). The cluster analysis reveals two main clusters: raw milk on the left with fewer compounds than mabisi on the right, which also has three sub-clusters for each batch apart from one sample from batch 3 fermented at 20 °C. These sub-clusters show slight variation of aroma compounds from one batch to another, which might be linked to differences in microbial composition at species level.

3.3. Effect of fermentation container type

3.3.1. Physicochemical properties

Three different batches of milk were simultaneously fermented at different temperatures in washed containers which, were previously used for tonga mabisi production with the objective to assess the effect of container type (used versus new). A much sharper decrease in pH was observed after 24 h in all samples fermented in used containers as compared to mabisi productions in which, new containers were used (see section 3.1 and section 3.2 and Fig. A2). To elaborate this, we selected the results of samples fermented at two temperatures: a low temperature of 20 °C (WT) and a high one of 25 °C (Fig. 7). Fig. 7a and d shows that the pH of the used container was lower than the new one throughout the fermentation at both temperatures. However, the differences between the used and new container were not significant ($p > 0.05$, Table A3). The rapid decrease in pH for samples fermented in used containers can be attributed to the formation of biofilms on the walls of the container during the previous fermentation, since the containers were only washed with hot water as practiced by the producers (Moonga et al., 2019). These biofilms potentially act as a starter culture and in turn accelerate the fermentation in the initial stages. The pH reduction should be further investigated using containers with a longer history of mabisi production.

A lower degree of syneresis was found for the used container as compared to the new one at both temperatures (Fig. 7c & f). The low degree of syneresis produced mabisi with thicker consistencies after 72 h of class 2 (7 cm) and class 3 at 20 °C and 25 °C, respectively compared to class 4 for both temperatures in the new container (Fig. 7c & f).

Generally, the results show that syneresis and consistency have an inverse correlation in mabisi, since a higher degree of syneresis leads to lower consistency. This was observed particularly for products with high syneresis of 48–52% which, resulted in mabisi with class 4 consistency (Table 1) of around 9 cm diameter of spread or more mainly for samples fermented at 25 °C and 30 °C after 72 h in the assessment of batch variation of mabisi production using both new and used containers

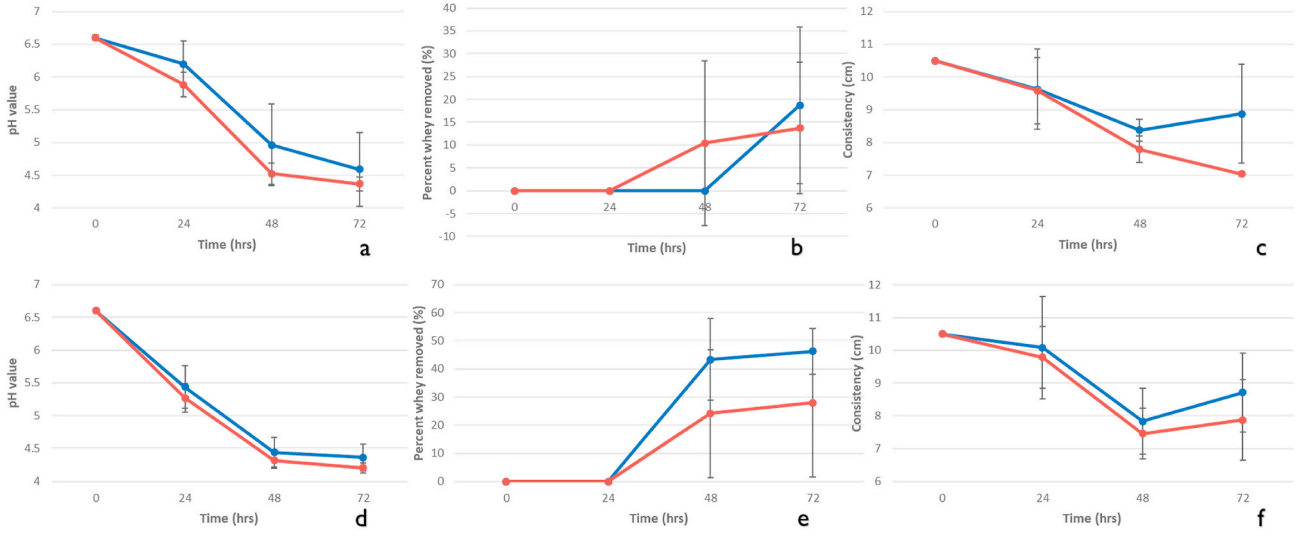


Fig. 7. Effect of temperature on physicochemical properties of tonga mabisi produced in new and used containers. The figure shows changes in: pH (panels a & d), degree of syneresis (panels b & e) and consistency with fermentation time (panels c & f) at temperatures of 20 °C and 25 °C, respectively. The colour blue is for a new container and red for a used container. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

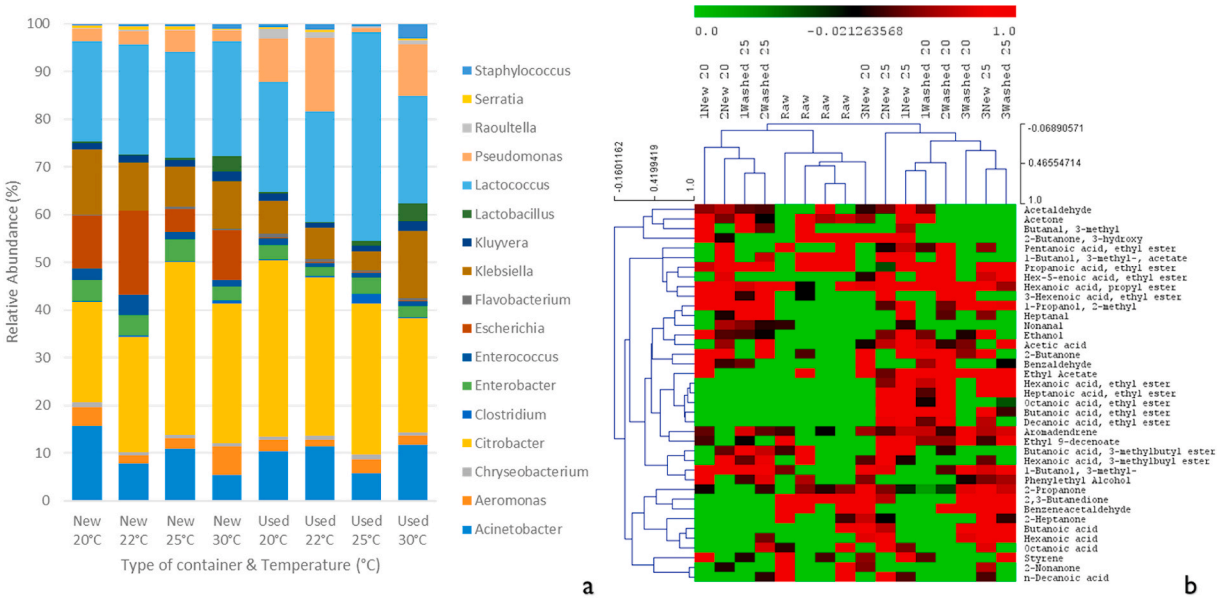


Fig. 8. Microbial composition and aroma compounds of mabisi production in new and used containers. Panel a. Relative abundance (%) of the top 20 microbes at genus level in mabisi fermented at four temperatures, 20°, 22°, 25° & 30 °C. Panel b. Heat map of volatile compounds from the same products with the colour green to red showing low to high levels of volatile compounds, respectively. Hierarchical clustering of the samples is also shown in the heat map. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(Fig. 3d & S2). A similar relationship between syneresis and consistency has also been observed by Bille et al. (2007) in *omasikwa*. In addition, to improve the consistency of another traditional product *amasi*, 40–50% of whey was removed (Mutukumira, 1995). The removal of whey is also practiced in the so-called "thick-tonga mabisi" (Moonga et al., 2019) though the proportion removed is unknown. Therefore, the present study has established that syneresis levels of not more than 30% produce mabisi with a desirable consistency of class 3 (see Fig. 7e and f).

3.3.2. Microbial community composition and aroma compounds

The composition of microbial communities of tonga mabisi produced in the used containers was also diverse (Fig. 8a) and dominated by species of the genus *Lactococcus* (22–44%) as well. *Lactobacillus* sp. were only observed in samples fermented at 30 °C with a relative abundance of 4%. The only difference between the mabisi produced in new and used containers was the higher proportions of members of the genera *Escherichia* and *Pseudomonas* in the former and latter containers, respectively. In general, tonga mabisi can be described as having a diverse microbial community composition regardless of fermentation temperature, different batches of production and use of new or used container over a three day fermentation period. Thus, the selected treatments did not affect the outcome of the fermentation in terms of microbial community composition.

The heat map shows that all raw milk samples were grouped in one sub-cluster with different and fewer aroma compounds than those found in mabisi (Fig. 8b). However, there is no clear distinction in the profile of aroma compounds found in mabisi produced in the new and used containers at 4 different temperatures.

3.4. Effect of production method

3.4.1. Physicochemical properties

Based on the observations of the effect of temperature on tonga mabisi, barotse mabisi was only incubated at two temperatures: 25° and 30 °C. As compared to the lower temperatures (20 °C and 22 °C), these temperatures produced a higher degree of syneresis in tonga mabisi (20–50%), which is important for the barotse mabisi production method as it involves the sequential removal of whey followed by re-addition of raw milk (Fig. 1b). Raw milk had a pH of 6.7, which dropped to pH 4.1 for samples incubated at 25° and pH 4.3 for those incubated at 30 °C after 3 days of fermentation (Fig. 9a). Thereafter, the pH of the samples incubated at 25 °C rose slightly (to pH 4.3) and the fermentation ended with the same pH value after a further three days of fermentation. However, for samples incubated at 30 °C, the pH dropped to 3.3 on the fifth day of fermentation and reached a final pH of 3.2 on the last day. The difference in the pH values for the final products was significant ($p < 0.05$, Table A4) with the barotse mabisi incubated at 30 °C producing a more acidic mabisi than the former. This pH was also much lower than

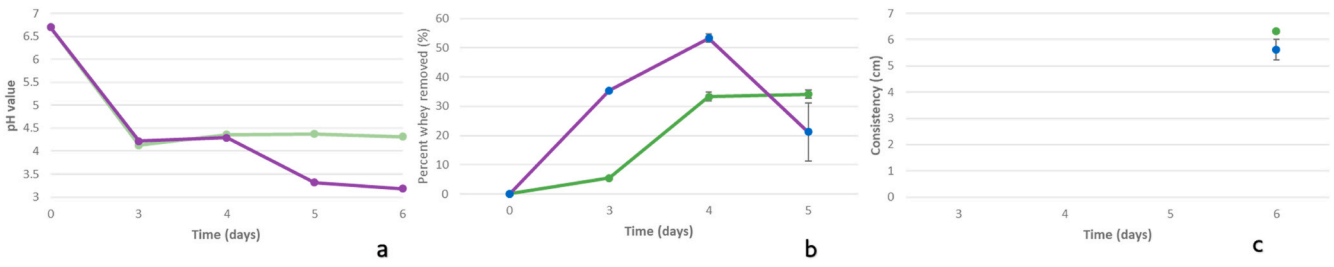


Fig. 9. Effect of temperature on physicochemical properties of barotse mabisi fermented at two temperatures. The figure shows changes in: (panel a) pH, (panel b) degree of syneresis and (panel c) consistency with fermentation time (hours). The colour green shows barotse mabisi incubated at 25 °C and purple those at 30 °C. The fermentation at each was done in triplicate. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

in both the single batch and multiple batch production of tonga mabisi, which had similar pH to barotse mabisi fermented at 25 °C.

Syneresis was observed after three days of fermentation and samples incubated at 30 °C had up to 35% in whey content at this stage, which further increased to a peak of 52% a day later and declined to 20% on the fifth day (Fig. 9b). On the other hand, samples fermented at 25 °C had low whey separation after three days with only 5% syneresis, which later increased sharply to 35% the following day and remained the same on the fifth day. Cumulatively, more whey was produced in the barotse mabisi fermented at 30 °C than at 25 °C, which implies that the product yield in volume terms was less for the production at 30 °C (48%) compared to 25 °C (58%). The degree of syneresis in barotse mabisi was comparable to the multiple batch production of tonga mabisi both in new and used containers (Figs. 5c & 7e).

Consistency was only measured at the end of fermentation (Fig. 9c). The barotse mabisi fermented at 25 °C had a class 2 consistency (6.3 cm) whereas the one fermented at 30 °C was thicker and had a class 1 consistency (5.6 cm). This is because the latter had a higher degree of syneresis and consequently, more whey was removed (52%) which was replaced with raw milk leading to an increase in the total solid content and thus, a "very thick" consistency. It must be stated that not all the whey could be removed during the production of barotse mabisi because of the design of the container which, retained a certain amount of whey. That generally, resulted in a final product having a slightly lower consistency than would be expected if a container, equipped with a better draining mechanism, was used. This shows that barotse mabisi had a thicker consistency than tonga mabisi (class 3–4) (Figs. 3d, 5d and 7c).

3.4.2. *Microbial community composition and aroma compounds*

The barotse mabisi (Fig. 10a) showed a different composition of the microbial communities as compared to tonga mabisi (Fig. 4a). At 25 °C, *Lactococcus* sp. dominated barotse mabisi while at 30 °C *Lactobacillus* sp. were dominant. *Lactococcus* had a relative abundance ranging from 84 to 91% when fermented at 25 °C and 6–9% at 30 °C whereas *Lactobacillus* had a range of 1–2% and 84–87%, at each of the two temperatures, respectively. There was a much larger proportion of *Lactococcus* (6–9%) in samples fermented at 30 °C than *Lactobacillus* (1%) in samples fermented at 25 °C. This suggests that during fermentation at 30 °C, *Lactococcus* was initially dominant as was the case in tonga mabisi but as the fermentation progressed, there was a shift towards *Lactobacillus* domination. This could be as a result of elevated temperature and low pH favouring the growth or survival of *Lactobacillus*. These results clearly show a significant effect of fermentation temperature on microbial community composition between two production methods and probably fermentation duration. The relative abundance of other microbes (non-LAB) in barotse mabisi was less than 10% whereas in tonga mabisi they accounted for 74% on average. These results are supported by recent findings from a survey of mabisi collected across Zambia which revealed that production method and pH influence the composition of bacterial communities (Moonga et al., 2020).

Fermentation temperature was found to have a pronounced effect on the composition of aroma compounds in barotse mabisi with two clusters clearly separated on basis of fermentation temperature (Fig. 10b). The samples fermented at 30 °C produced more compounds than those fermented at 25 °C which, include 12 esters, 2 alcohols, 2 fatty acids, 1 terpene, 6 ketones, and 5 aldehydes that were in low relative abundance

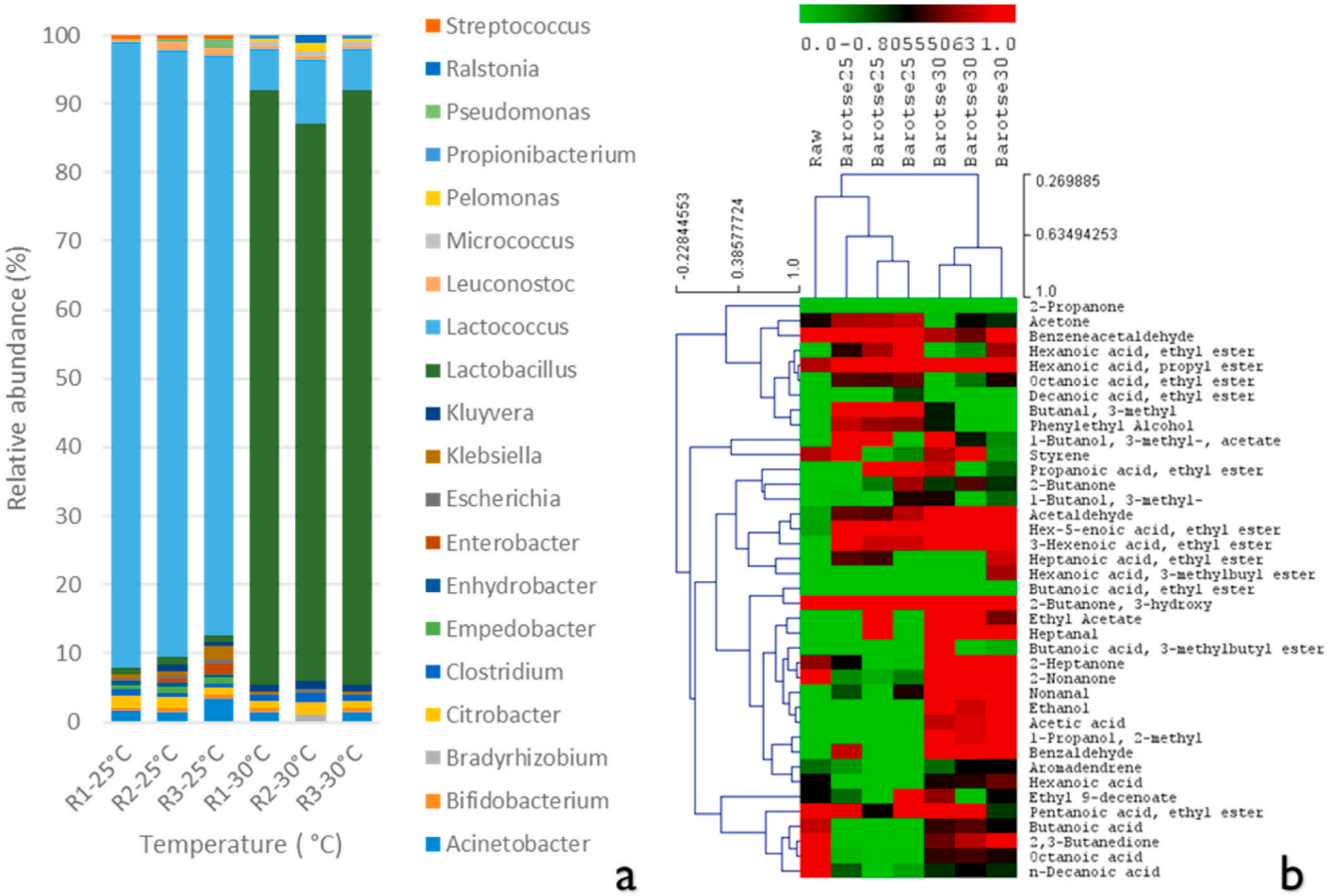


Fig. 10. Microbial composition and aroma compounds of barotse mabisi fermented at two temperatures. Panel a. Relative abundance (%) of the top 20 microbes at genus level in mabisi fermented at two temperatures, 25° & 30 °C. Panel b. Heat map of volatile compounds from the same product with the colour green to red showing low to high levels of volatile compounds, respectively. Hierarchical clustering of the samples is also shown in the heat map. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

in samples fermented at 25 °C. This difference is most likely is caused by the differences in the composition of the microbial community occurring at the two incubation temperatures.

4. Conclusion

The aims of this study were to evaluate the effects of fermentation temperature on key quality parameters and on microbial community composition of two types of mabisi. Overall, we found that fermentation temperature did not show a clear effect on the composition of microbial communities of tonga mabisi, which retained a diverse and stable microbial community regardless whether the production was from a single batch or multiple batches of raw milk. Also use of new containers or previously used containers did not lead to clear differences in the complex composition of the microbial community. In tonga mabisi, *Lactococcus* was the dominant genus. Interestingly, temperature had a clear effect on microbial community composition in barotse mabisi with the mesophilic *Lactococcus* dominating the fermentation at 25 °C and the thermophilic *Lactobacillus* dominating at 30 °C. Furthermore, more aroma compounds were produced at 30 °C in barotse mabisi than at 25 °C whereas tonga mabisi retained a similar aroma profiles regardless of temperature or treatment.

Temperature exerted a profound effect on fermentation time of tonga mabisi. Furthermore, used containers also accelerated fermentation whereas new containers coupled with low ambient temperatures slowed down fermentation up to three days. For this study, a classification system for mabisi consistency was developed with four classes where the thicker mabisi (barotse) was in class 1 and 2 and tonga mabisi was in class 3 and 4. However, there is need to validate these classes as well as the acidic taste levels with a sensory evaluation.

CRediT authorship contribution statement

Himoonga Bernard Moonga: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Sijmen E. Schoustra:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Software, Supervision, Writing - review & editing. **Anita R. Linnemann:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing - review & editing. **Joost van den Heuvel:** Data curation, Formal analysis, Software, Validation, Writing - review & editing. **John Shindano:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing - review & editing. **Eddy J. Smid:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Software, Supervision, Writing - review & editing.

Declaration of competing interest

None to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2020.110350.

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