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Effect of Sorghum and Maize Malt Particle Size on Physicochemical, Stability, Microbiological, and Sensory Characteristics of Umqombothi

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Abstract: The consumption of Umqombothi alcoholic beverages in South Africa is widespread in townships and rural areas. This study aimed to investigate the effect of sorghum and maize malt particle size on the physicochemical, microbiological, stability, and sensory characteristics of Umqombothi. Three different particle sizes were compared, namely control, coarse, and fine powder particle sizes. Subsamples were taken at the following stages, during the production of Umqombothi: first, second, third, fourth, and fifth. Lactic acid bacteria were significantly dominant, with 8.16, 7.11, and 5.91 log cfu/mL in the final product for the control, coarse, and fine powder particle sizes. The yeast counts were 3.3, 4.64, and 5.63 log cfu/mL for the control, coarse, and fine powder particle sizes. Molds were significantly reduced to non-detectable levels after the second fermentation and the total coliforms in the final product were reduced for all particle sizes. The total soluble solids significantly decreased in the second fermentation samples. The coarse particle size exhibited significantly higher alcohol and significantly lower pH levels, which are encouraging signs for improved quality and shelf life. Different particle sizes of sorghum and maize malt affect the quality of the finished product and the production method of umqombothi.



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Keywords: umqombothi; fermentation; particle sizes; lactic acid bacteria; traditional beer

1. Introduction

Indigenous drinks, also known as traditional beverages, are created by the inhabitants of a particular region, utilizing old techniques and locally farmed and sourced ingredients [1]. Traditional beverages from South Africa, for which maize or sorghum are valuable raw materials, are known as Umqombothi [2]. Starch is a significant component in Umqombothi production, as it serves as a carbohydrate source and as a thickening and suspending agent. These particles are suspended through starch gelatinization, making the beverage creamy. However, the complete conversion of starch is avoided [3–5]. Also, the Umqombothi fermentation process is traditionally performed under uncontrolled conditions, adding to the challenge of producing a beverage with an extended shelf life [4,5]. The production of Umqombothi typically involves lactic acid and alcoholic fermentation. Due to their higher growth rate, lactic acid bacteria (LAB) fermentation dominates the early stages. At the same time, yeast gradually builds up in the latter part of the fermentation process [6–9]. While it remains a traditional process, the brewing of Umqombothi has benefited significantly from enhanced scientific knowledge and technology over the last century. The modern malting and brewing industry has pioneered various technical, biochemical, microbiological, and genetic inventions [10]. Transgenic malts and appropriate starter cultures in malting offer intriguing new possibilities for ensuring balanced enzyme activity and avoiding harmful fusarium contamination [11]. Several genetically modified brewer's yeasts have been developed, such as those encoding -acetolactate decarboxylase

and super-flocculating yeasts [11]. These and other advancements generally fail to adequately address the effect of malt milling on particle sizes and their impact on the quality of Umqombothi.

In contrast to other traditional African beers, sorghum and maize malt are not milled or graded during the Umqombothi production process [10,12]. Research must be conducted on the effects of varying particle sizes in the production of Umqombothi. Milling reduces particle sizes to the micron level, which enhances the carbohydrate solubility yield by releasing several enzymes; milling causes the physical breakdown of ingredients, which liberates soluble carbohydrates, without the need for external enzymatic treatment. Starch hydrolysis is greatly influenced by its physical state. The degradation of starch increases water absorption and enzymatic sensitivity, influencing the physicochemical qualities of starch as well [10,12].

Enzymes attach easier to amorphous starch regions, than crystalline starch regions. Enzymatic amylolysis of tiny and large starch granules occurs in different ways. Distinct sizes of starch granules have variable structures in terms of amylose and amylopectin ratios, resulting in different physicochemical qualities [10]. From the surface to the centre of the starch granule, amylopectin and amylose have a distinct structure that ranges from small and medium to large granules [10,13]. The most commonly used enzymes in brewing for the hydrolysis of wort are α -amylase, β -amylase, and endo- β -1,3:1,4glucanases [10,14]. Because sorghum beverages have played a significant role in traditional civilization, it is critical to understand the impact of particle size on malts. However, the number of studies conducted in this area is limited, especially information on the effect of particle sizes on the production of Umqombothi. This study aimed to evaluate the effect of particle size on the physiochemical, microbiological, stability, and sensory characteristics of Umqombothi. It is hypothesized that reducing the particle size of the malt will improve the stability and sensory characteristics of Umqombothi.

2. Materials and Methods

2.1. Source of Material and Equipment

A nearby retailer in Bellville, South Africa, provided the red sorghum, maize malt, and beer powder. Boxer Supermarket sold maize malt in East London, South Africa (Umthombo wombona). The Neogen culture media obtained from Lasac South Africa included plate count agar (NCM0010A), de Man–Rogosa–Sharpe agar (NCM0190A), violet red bile agar (bio lab, Merck, South Africa), and Rose Bengal Chloramphenicol agar (NCM0135A). Umqombothi produced in a lab, with a coarse particle size and fermentation temperatures of between 30 and 30 °C, was subjected to a sensory evaluation. In Cape Town, Paarl, in the Drakenstein Municipality, Umqombothi was purchased in Mbekweni (Langabuya, South Africa) and New-Rest (Ezimbacwini, South Africa) townships. Every piece of brewing equipment needed to produce Umqombothi was purchased from the Cape Peninsula University of Technology (CPUT) in Cape Town, South Africa, by the Department of Food Science and Technology.

2.2. Milling of Sorghum and Maize Malt

A Stake STR100AU (Crankendstein made in South Africa) two-roll mill with fluted rollers was used to grind red sorghum and maize malts into two different particle sizes: fine powder and coarse powder. The lab-scale roller system was used to determine the setup and settings of the roll mill using pre-trials [15]. An Allen key was used to adjust the roller gap for the red sorghum and maize malt particle sizes, which were 1 mm for the coarse size and 0.5 mm for the fine powder. Sorghum and maize malts with fine and coarse powder particles were developed. The samples were stored at room temperature.

2.3. The Umqombothi Production Process

The Umqombothi production flow diagram employed in this chapter is illustrated in Figure 1. Weighing out five hundred grams of maize meal, one hundred grams of

red sorghum malt (control, coarse, and fine powder; ingredients as listed on the original package), and one hundred grams of maize malt (control, coarse, and fine powder), in three different containers, labelled control, coarse, and fine powder particle size, allowed us to produce three batches of umqombothi. After that, each container received 5 L of warm water (thirty-five degrees Celsius), which was added and blended to create a uniform mixture. The initial spontaneous fermentation occurred in all three batches after a 24 h incubation period at 30 °C. The three batches were divided into the sediment and the supernatant after 24 h. The supernatant from each batch was poured into a different pot made of stainless steel and brought to the boil. Sediments from each batch were incorporated into the boiling water. After that, the temperature was lowered to sixty degrees Celsius and stirred for forty minutes, until something akin to porridge developed. Three hundred grams of red sorghum malt (control, coarse, and fine powder) and seventy-five grams of Mnanti beer powder were added as an inoculum after the porridge had cooled to room temperature. After that, the mixture was incubated for twenty-four hours at twenty-five degrees Celsius. For the second fermentation, the three batches were incubated at 30 °C for a whole day. After that, the products were removed from the incubation chamber and put through a filter, with a pore size of about 0.55 mm. Each batch's Umqombothi supernatant was moved to a marked sterile container, and the sediment was thrown away [16].

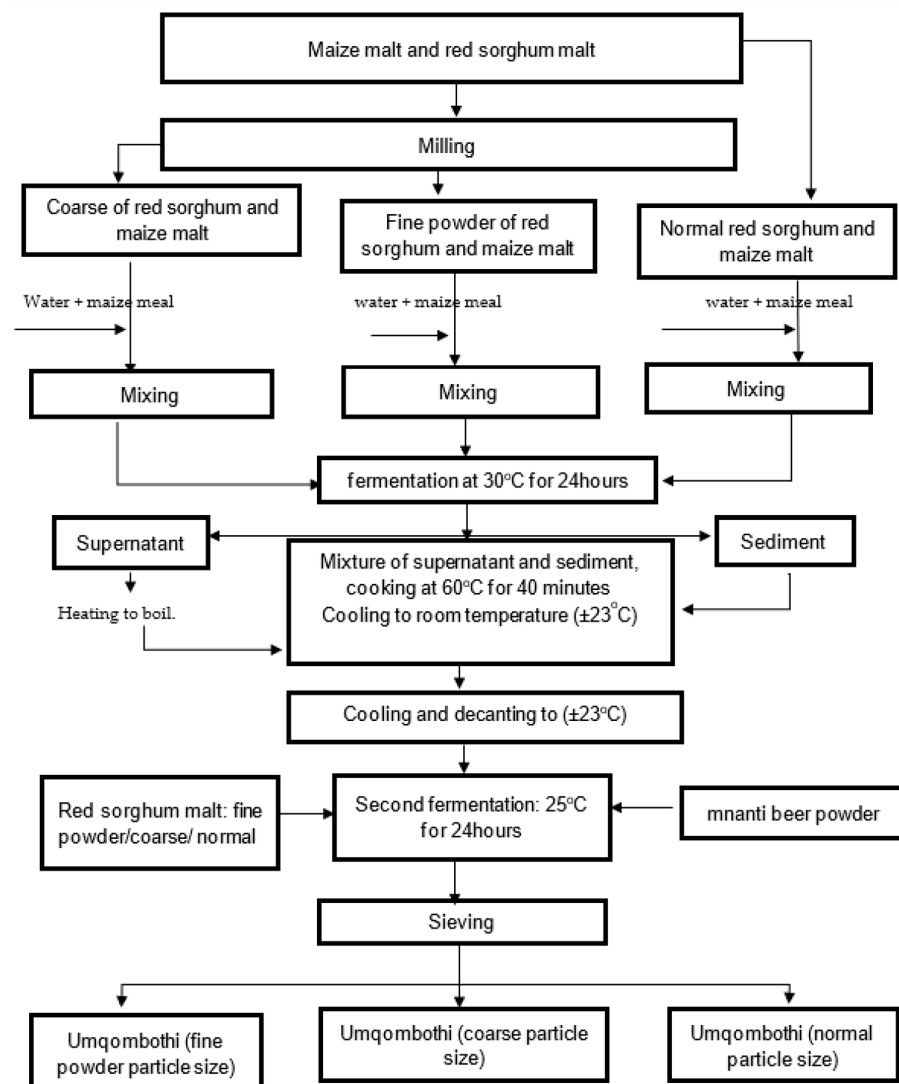


Figure 1. Flow diagram of the process to produce Umqombothi from different particle sizes (control, coarse, and fine powder) of sorghum and maize malt.

2.4. Sampling

The sample before the first fermentation (1st), after the first fermentation (2nd), before the second fermentation (3rd), after the second fermentation (4th), and the final product (5th), were sampled in triplicate for every batch. Before being analyzed, the samples were taken using a sterile sampling cup and kept chilled at 4–6 °C. Chemical, physicochemical, microbiological, and sensory analyses were performed on the samples. For every batch, samples of the finished product were subjected to sensory analysis.

2.5. Chemical Analysis

2.5.1. Analysis of the pH of Umqombothi

At room temperature (23 ± 2 °C), the pH of every sample was determined with a calibrated pH meter from Mettler-Toledo GmbH, Greifensee, Switzerland (FiveEasy F20) [17].

2.5.2. Determination of Total Soluble Solids (TSSs) and Specific Gravity (SG) in Umqombothi

A refractometer (Bellingham & Stanley, Nottingham, UK) measured the total soluble solids. A Brewcraft gravity refractometer was used to test the gravity. The material was dropped many times onto the prism's surface. There should not be any bubbles or material particles in the liquid on the prism. After that, the prism was shut. The device was oriented towards the light to obtain an accurate readout. The eyepiece was focused until a sharp picture appeared, if needed. The percentage of the total soluble solids reading was determined by the point on the vertical scale, where the demarcation line and dark areas intersect [17].

2.5.3. Determination of the Alcohol Content during the Umqombothi Production Process

Ten milliliters of each sample were centrifuged using an Avanti J-E Centrifuge (Beckman Culture, Brea, CA, USA) at 11,000 rpm for ten minutes. The alcohol concentration was determined using gas chromatography (GC) and the supernatants obtained from each sample. The procedures in [16] were followed. Agilent Technologies' Gas Chromatograph System 7890A was used. For the calibration, 96% ethanol was diluted to a concentration 2%, 4%, 6%, 8%, and 10%, with a correlation of 0.99.

2.6. Physical Characteristics of Umqombothi

2.6.1. Color Measurements of the Umqombothi

Using a Konica Minolta Spectrometer CM 5 [Norich (pty) Ltd], with 45°/0° standards, set at the standard observer 10° and D65, the color of the Umqombothi was assessed. A black tile ($L^* = 5.49$, $a^* = 7.08$, $b^* = 4.66$) and a white tile ($L^* = 93.41$, $a^* = 1.18$, $b^* = 0.75$) were used to zero calibrate the instrument. A light-colored sample container was filled with 3 g of Umqombothi, and the reflection was measured using the L^* a^* b^* and LCh color scales. Lightness is represented by the L^* coordinate, where 0 is blackness, and 100 is brightness. Coordinates a^* and b^* denote the green (−)/red (+) and blue (−)/yellow (+) chromatic, respectively. Each sample was measured in triplicate, with $0^\circ = +a^*$, $90^\circ = +b^*$, $180^\circ = -a^*$, and $270^\circ = -b^*$ for the C^* (chroma) and h (hue) angles. Using Equation (1), the overall color difference of the Umqombothi (ΔE^*) was determined [18].

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (1)$$

2.6.2. Measuring the Syneresis (STS) of Umqombothi

The Umqombothi samples were stored at 4 °C for two to three days, before being subjected to the Samson A [19] technique to assess the syneresis. An Avanti J-E Centrifuge (Beckman Culture, Brea, CA, USA) was used to centrifuge the materials for 10 min at 4 °C,

with a weight of 350 g. Once the triplicate analysis was completed, the syneresis (STS) was computed using the following equation:

$$\%STS = \frac{V_1}{V_2} \times 100 \quad (2)$$

V_1 = Volume of Umqombothi whey collected after drainage

V_2 = Volume of Umqombothi beer

2.6.3. Determination of the Viscosity of Umqombothi Traditional Beer

The viscosity of Umqombothi was measured using a Rheolab QC viscometer (Anton Paar, Graz, Austria, 81602957), with a temperature device C-PTD 180/AIR/QC, 81622948, and a measurement system CC27 (Austria), during the before the first fermentation (BFF) and after the second fermentation (ASF) sample stages over time. Following the manufacturer's instructions, the beverage (18 mL) was put into an upward projected sample cup and examined for 17 min at 25 °C and 4 °C. The shear rate (s⁻¹) remained constant during each run. Three repeats of the viscosity analysis were carried out [20].

2.7. Microbiological Analysis

2.7.1. Enumeration of the Microorganisms in Umqombothi

The microorganisms in Umqombothi were counted using the procedures outlined in SANAS 4833:2007 [21] and ISO: 4833:2007 [22]. The microorganisms in Umqombothi were counted using the pour plate method. A colony counter from Gerber Labortechnik (Berlin, Germany) was used to count all the typical colonies. Positive controls were achieved by streaking with *Escherichia coli*, *Lactobacillus gasseri*, yeast (*Saccharomyces cerevisiae*), mold (*Aspergillus* spp.), and bacteria. One milliliter of the Ringer solution in a stomacher bag was used as the control and, after that, PCA was added to the plates. The plates were incubated for 24 h at thirty-seven degrees Celsius. There were three duplicates of each experiment run. Only the plates with 30 to 300 colonies were tallied [18].

2.7.2. Bacterial Enumeration

A total of 10 g of Umqombothi was weighed and mixed with 90 mL of sterile Ringer's solution (10⁻¹). After that, further dilutions (10⁻¹ to 10⁻⁶) were made. A one milliliter aliquot for each dilution was meticulously and aseptically placed into the base of four marked sterile Petri dishes. Approximately 12–15 mL of de Man–Rogosa–Sharpe agar, violet red bile agar, and cooled plate count agar, were added to each Petri dish for each dilution. The plates were then gently spun to ensure thorough mixing. All the plates were incubated upside-down, after being given time to harden. For 48 h, the MRS, PCA, and VRBA plates, for every dilution, were incubated at thirty-seven degrees Celsius [16].

2.7.3. Enumeration of Yeast and Molds

Following the sequence of dilutions in Section 2.7.2, a 1 mL aliquot was aseptically transferred to sterile Petri dishes, which were previously labelled. Next, twelve to fifteen milliliters of cooled Rose Bengal Chloramphenicol agar were added to every Petri dish, and the mixture was mixed by swirling the plate. After solidification, the plates were inverted and incubated at 25 °C for five days [16].

2.8. Sensory Evaluation

The IFST Guidelines for Ethical and Professional Practices for the Sensory Analysis of Foods were used as the basis for the sensory evaluation. Before the sensory evaluation, Umqombothi was deemed safe after being evaluated for any microbiological, chemical, and physical dangers. The ingredients and production methods are the same as those used to make commercial Umqombothi. The scope of the research was limited to the activities specified in the proposal. The test subjects were volunteers and they were advised of their ability to withdraw from the study. The research subjects also provided their informed

consent. The participants were told about the product's ingredients and alcohol level, and the participants' privacy and confidentiality were safeguarded. Umqombothi is a traditional alcoholic beverage from South Africa, with two to three per cent alcohol by volume. Owing to the potential health risks associated with alcohol consumption and its detrimental effects on driving skills, the panelists were only allowed to participate in one tasting round. Each participant was given 90 mL of Umqombothi, or approximately 1.8 milliliters of ethanol. The participants were also informed that tasting, as opposed to drinking, was the aim. Thus, they were only obliged to consume part of the beverage.

Fifty unskilled panelists participated in a consumer sensory study of the three batches of Umqombothi at the Cape Peninsula University of Technology's Sensory Laboratory, which is part of the DFST. The samples were arranged in parallel, fifty milliliter white polystyrene cups, on a plastic tray. A three-digit number was used to identify each sample cup with 30 mL of the corresponding Umqombothi batches, which were delivered at room temperature (23 ± 2 °C). A cup of water was offered to help with palate cleansing both before and during tastings. As in [23], a score sheet comprising three coded samples and a 5-point hedonic scale—with 1 denoting extreme hatred and 5 denoting extreme like—was presented to the participants. A 5-point hedonic rating system was provided to the participants, so they could assess each item on its own merits, considering the appearance, color, taste, aroma, texture, and overall acceptability. For the further manufacturing of Umqombothi, the batch with the preferred particle size determined by the sensory assessment was employed [17].

2.9. Data Analysis

Multivariate analysis of variance (MANOVA) was used to determine the significant differences ($p < 0.05$) in attributes among the samples. Duncan's multiple range tests were used to separate the mean where a significant ($p \leq 0.05$) difference existed (IBM SPSS version 22, 2013).

3. Results

3.1. Effect of Particle Size on the Physicochemical Characteristics of Umqombothi

There were five separate sample phases in the Umqombothi manufacturing process, namely before the first fermentation (first sample), after the first fermentation (second sample), before the second fermentation (third sample), after the second fermentation (fourth sample) and, lastly, the final product (fifth sample).

Table 1 displays the pH that was attained throughout the Umqombothi production process. There was a significant ($p < 0.05$) difference in the pH between the sampling stages for the control particle sizes, except for the second and fifth sample stages. The significant ($p < 0.05$) decrease in pH is due to a significant increase in LAB during the Umqombothi production process, specifically in the first and the fifth stages for the control, coarse, and fine powder particle sizes. The lactic acid bacteria produced lactic acid and acetic acid, causing the pH to decrease significantly. The pH for the control, coarse, and fine powder particle sizes align with previous results reported for Umqombothi [9,16,24]. The current study confirmed that the particle size influenced the pH of Umqombothi during the production process. The lower the particle size, the higher the pH level during the fifth sampling stage. Beverages with a significantly low pH last longer in storage, have superior safety and quality, and have antimicrobial properties.

The beverages with a low pH assist in the removal of harmful microorganisms that may constitute a health risk [25]. When contrasting how the fermentation temperature affects the pH, there was no significant difference between the overall particle sizes on the pH of coarse (4.08) and fine powder (4.07). The control particle size was significantly ($p < 4.13$) higher than the coarse and fine powder particle sizes. According to [9], beverages with a lower pH (4.07), such as those with fine powder/coarse powder particle sizes, offer better quality and safety, an extended shelf life, and stronger antibacterial qualities. The overall pH for the control, coarse, and fine powder particle sizes, respectively, are higher

than the pH of 3.67 reported by [16,24] on the final Umqombothi product. This could be due to the fermentation period, or the source of the grains and inoculum used.

Table 1. Effect of particle size on Umqombothi's pH level *.

Sampling Stage	pH		
	Control	Coarse Powder	Fine Powder
First sample	6.03 ± 0.01 ^a	6.02 ± 0.01 ^a	5.96 ± 0.01 ^a
Second sample	3.55 ± 0.02 ^b	3.41 ± 0.01 ^b	3.46 ± 0.01 ^b
Third sample	4.07 ± 0.02 ^c	4.02 ± 0.01 ^c	3.97 ± 0.01 ^c
Fourth sample	3.54 ± 0.01 ^b	3.47 ± 0.01 ^d	3.48 ± 0.01 ^d
Fifth sample	3.45 ± 0.00 ^e	3.46 ± 0.01 ^d	3.49 ± 0.00 ^d
Overall particle size	4.13 ± 1.01 ^a	4.08 ± 1.03 ^b	4.07 ± 0.99 ^b

* The determination of the mean value ± standard deviation in triplicate. There is a substantial ($p \leq 0.05$) difference in the mean values in the same column separated by distinct characters (different superscript letters). Before the first fermentation (first sample), after the first fermentation (second sample), before the second fermentation (third sample), after the second fermentation (fourth sample) and, lastly, the final product (fifth sample).

Umqombothi has a moisture content of 94.67% and a limited shelf life of 1–3 days, according to [24]. Foods with high moisture content are more prone to microbial development, reducing its shelf life. The effect of excessive moisture on the shelf life of Burukutu, Ghana's traditional beverage, was studied by [26]. Sorghum fermentation usually activates enzymes, decreases the pH, and increases metabolic and microbial activity. This causes the breakdown of starch, enhancing the nutritional quality [9]. The activity of enzymes is affected by the pH, which is essential in liquefaction and the conversion of malt starch into fermentable sugars [24]. Furthermore, an elevated pH promotes microbial growth by lowering proteinase and amylase activity and stability [27].

Table 2 shows the influence of different particle sizes on the TSSs, during the making of Umqombothi. There was a substantial ($p < 0.05$) variation in the control, coarse, and fine powder particle sizes, between all the sample phases. As the starch granules gelatinize, the endosperm protein that encircles them becomes softer, which results in the transfer of the grain into the retting water and a subsequent increase in the concentration of total soluble solids. This could explain why the TSSs increase during the fermentation and third sampling stages. Cooking the sour porridge long enough allows the starch to gelatinize and the yeast cell's lockup nutrients to be released. The TSSs level is influenced by the cooking time and particle size [28]. As the second fermentation stage progresses, the accessible solids are utilized by microorganisms, such as yeast, to create alcohol and an aroma [17]. Significant ($p < 0.05$) decreases in the TSSs were observed in the final products with the control, coarse, and fine powder particle sizes, possibly due to sieving. By removing the second conversion of malt, [29] a similar trend was observed. When the samples were held between 28 °C and 30 °C, [17] a decrease in the TSSs from 9.7 to 8 was observed. According to [17], the greatest reduction in the TSSs resulted in a more significant bacterial burden.

Table 2. Effect of particle size on Umqombothi total soluble solids values *.

Sampling Stages	Total Soluble Solids (°Brix)		
	Control	Coarse Powder	Fine Powder
First sample	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
Second sample	1.10 ± 0.00 ^b	1.80 ± 0.00 ^b	1.87 ± 0.12 ^b
Third sample	9.83 ± 0.06 ^c	9.93 ± 0.12 ^c	10.00 ± 0.00 ^c
Fourth sample	4.67 ± 0.29 ^d	5.67 ± 0.29 ^d	6.00 ± 0.00 ^d
Fifth sample	4.50 ± 0.00 ^e	5.50 ± 0.00 ^e	5.83 ± 0.29 ^e
Overall particle size	4.02 ± 3.56 ^a	4.58 ± 3.57 ^b	4.74 ± 3.62 ^c

* The determination of the mean value ± standard deviation in triplicate. There is a substantial ($p \leq 0.05$) difference in the mean values in the same column separated by distinct characters (different superscript letters). Before the first fermentation (first sample), after the first fermentation (second sample), before the second fermentation (third sample), after the second fermentation (fourth sample) and, lastly, the final product (fifth sample).

The overall TSSs differed considerably ($p < 0.05$) for the control, coarse, and fine powder particle sizes. Umqombothi produced with the fine powder particle size had a significantly greater amount of (4.74) TSSs than the coarse powder and normal particle sizes. Not all the dissolved solids were available for utilization by the microorganisms present, as seen in the levelling of the solids in the products at the end of the production process. The levelling may be due to the inhibiting effects of ethanol.

During gelatinization, the amount of TSSs increases, transferring the grain into the retting water [27]. This might account for the increasing trend in the TSS levels, which coincides with the fermentation and heating of the fine powder particle size. Fine powder is the perfect particle size when it comes to TSSs.

Table 3 shows the specific gravity (SG), as influenced by various particle sizes, during the manufacturing of Umqombothi. Specific gravity is a measurement that indicates the progress of the fermentation process [30]. The more sugar dissolved in the wort, the greater the SG value. The overall particle sizes and specific gravity levels of the control, coarse, and fine powder particle sizes did not differ significantly. The lower the particle size, the higher the SG at the fifth sampling stage. The proportion (%ABV) of alcohol by volume of the resulting beverage can be calculated by subtracting the original gravity from the final gravity of the wort [31,32]. The increase in the SG, from unfermented to fermented products, followed a similar pattern to that described by [26].

Table 3. The impact of particle size on Umqombothi specific gravity levels *.

Sampling Stages	Specific Gravity		
	Control	Coarse Powder	Fine Powder
First sample	1.00 ± 0.00 ^a	1.00 ± 0.00 ^a	1.00 ± 0.00 ^a
Second sample	1.04 ± 0.05 ^a	1.01 ± 0.00 ^a	1.01 ± 0.00 ^a
Third sample	1.04 ± 0.00 ^a	1.04 ± 0.00 ^a	1.04 ± 0.00 ^a
Fourth sample	1.02 ± 0.00 ^a	1.03 ± 0.00 ^a	1.03 ± 0.00 ^a
Fifth sample	1.02 ± 0.00 ^a	1.11 ± 0.12 ^b	1.28 ± 0.00 ^b
Overall particle size	1.02 ± 0.02 ^a	1.04 ± 0.06 ^a	1.07 ± 0.11 ^a

* The determination of the mean value ± standard deviation in triplicate. There is a substantial ($p \leq 0.05$) difference in the mean values in the same column separated by distinct characters (different superscript letters). Before the first fermentation (first sample), after the first fermentation (second sample), before the second fermentation (third sample), after the second fermentation (fourth sample) and, lastly, the final product (fifth sample).

Table 4 shows the alcohol concentration as influenced by various particle sizes throughout the production of Umqombothi. The fifth sampling stage with the coarse powder (3.44%) particle size exhibited the ($p < 3.44$) highest alcohol content in comparison with the fine powder (3.28%) and control (2.00%) particle sizes, respectively. The study confirmed that particle size does affect the alcohol content [10]. The control particle size (non-milled ingredients, used as they come from the manufacture's package) resulted in a low % of alcohol. In contrast, the coarse and fine powder particle sizes produced Umqombothi with a higher % of alcohol.

The % of alcohol content contributes to the beverage's flavor and impacts its quality [33]. The alcohol levels in the final sampling stages for all three particle sizes correspond to those reported by [9], i.e., between 2–3.5%.

The overall values of the alcohol content of Umqombothi, as affected by the particle sizes of the control, coarse, and fine powder, were significantly ($p < 0.05$) different. The coarse powder particle size exhibited the ($p < 1.44$) highest % of alcohol than the fine powder and control particle sizes, respectively. This can be ascribed to the available solids utilized by yeast and LAB and the subsequent formation as the fermentation process progresses [26]. The reduction in the % of the alcohol content may be related to evaporative ethanol loss before the second fermentation stage due to cooking [28]. The overall % alcohol levels for the particle sizes recorded in this study were 1.5% lower than those reported by [9] (2–3.5%) and [16] (2.6%). The location and method of brewing will affect Umqombothi's ethanol

content [9,34]. According to [3], the alcohol content ranges from 1–8%, but the most typical range is 2.5–4.5.

Table 4. The effect of particle size on the % alcohol content of Umqombothi *.

Sampling Stages	Alcohol (%)		
	Control	Coarse Powder	Fine Powder
First sample	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
Second sample	0.20 ± 0.00 ^b	0.33 ± 0.00 ^b	0.38 ± 0.00 ^b
Third sample	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
Fourth sample	2.00 ± 0.00 ^c	3.44 ± 0.00 ^c	3.28 ± 0.00 ^c
Fifth sample	2.00 ± 0.00 ^c	3.44 ± 0.00 ^c	3.28 ± 0.00 ^c
Overall particle size	0.84 ± 0.98 ^a	1.44 ± 1.69 ^b	1.39 ± 1.61 ^c

* The determination of the mean value ± standard deviation in triplicate. There is a substantial ($p \leq 0.05$) difference in the mean values in the same column separated by distinct characters (different superscript letters). Before the first fermentation (first sample), after the first fermentation (second sample), before the second fermentation (third sample), after the second fermentation (fourth sample) and, lastly, the final product (fifth sample).

According to [31], the ethanol levels may decline with the increasing age of the beverage, due to microbial conversion of alcohol to acetic acid. Beverages with an alcohol level of 1–2% and 0.5% are commonly classed as low-alcohol and non-alcohol beers [35] and there is great demand for these brews in the worldwide beverage market. This new trend has evolved due to greater consumer awareness of the negative health impacts of alcohol consumption. Product inhibition is common during simultaneous saccharification and fermentation, as ethanol, a fermentation product, inhibits zymase, and saccharification products inhibit hydrolytic enzymes [27]. In view of all of that, coarse powder is the best particle size in this regard for Umqombothi production.

3.2. Color Characteristics of Umqombothi

Umqombothi's color characteristics, as impacted by fermentation and particle size, namely lightness (L^*), greenness ($-a^*$), redness ($+a^*$), blueness ($-b^*$), and yellowness ($+b^*$), are presented in Table 5.

Table 5. The effect of particle size on the lightness value of Umqombothi *.

Sampling Stages	Lightness		
	Control	Coarse Powder	Fine Powder
First sample	92.60 ± 0.00 ^a	88.90 ± 0.00 ^a	87.18 ± 0.00 ^a
Second sample	91.80 ± 4.9 ^a	93.60 ± 0.00 ^b	92.13 ± 0.00 ^b
Third sample	60.59 ± 0.00 ^b	60.40 ± 0.00 ^c	49.44 ± 0.00 ^c
Fourth sample	66.46 ± 0.08 ^c	66.69 ± 0.46 ^d	98.76 ± 0.00 ^d
Final product (FP)	67.19 ± 0.03 ^c	62.21 ± 0.05 ^e	61.95 ± 0.10 ^e
Overall particle size	75.73 ± 14.24 ^a	74.36 ± 14.52 ^b	77.89 ± 19.57 ^c

* The determination of the mean value ± standard deviation in triplicate. There is a substantial ($p \leq 0.05$) difference in the mean values in the same column separated by distinct characters (different superscript letters). Before the first fermentation (first sample), after the first fermentation (second sample), before the second fermentation (third sample), after the second fermentation (fourth sample) and, lastly, the final product (fifth sample).

Umqombothi's lightness, obtained from the different particle sizes at different sampling stages, is presented in Table 5. During cooking (third stage), starch is metabolized by yeast and LAB into simple sugars. It is further converted into ethanol and CO₂, which could cause a significant intensification in the product's lightness [36]. Before the second fermentation, it exhibited the lowest lightness levels compared to all the other sampling stages for the control, coarse, and fine powder particle sizes; at this stage, which involves an increase in temperature, starch is converted into simple sugars, and the mixture becomes

thick and dark. During the preparation of Umqombothi, prolonged starch breakdown is avoided in the third sampling stage, since it might result in an overly thin beer [5,9].

The overall lightness levels were considerably ($p < 0.05$) different for the control, coarse, and fine powder particle sizes. Umqombothi produced with a fine powder particle size had a considerably ($p < 0.05$) greater lightness than the control and coarse powder particle sizes, as the values were much closer to 100. Milling decreases the particle size to the micron level, which increases the carbohydrate solubility yield by employing multiple enzymes that liberate soluble carbohydrates, without the need for enzymatic treatment. Starch degradation improves water absorption and enzymatic sensitivity [10,12]. According to [15], the sorghum lightness (L^* value) increased with a decreasing particle size, whereas in the final Umqombothi product (FP) the opposite effect was observed.

The redness of Umqombothi at different sample phases and with different particle sizes is shown in Table 6. Umqombothi is within the redness color space, as indicated by its positive redness values. Given that sorghum grain first appears to be reddish, this was anticipated [15]. Physical breakdown occurs during milling, releasing soluble carbohydrates without the need for enzymes [10]. The overall redness levels, as affected by the particle sizes, were 3.19, 3.59, and 3.42, and they were considerably ($p < 0.05$) distinct for the control, coarse, and fine powder particle sizes.

Table 6. The impact of different particle size on Umqombothi redness levels *.

Sampling Stages	Redness			Overall Sampling Stages
	Control	Coarse Powder	Fine Powder	
First sample	0.77 ± 0.00 ^a	0.89 ± 0.00 ^a	0.96 ± 0.00 ^a	0.87 ± 0.83 ^a
Second sample	0.64 ± 0.29 ^a	0.22 ± 0.00 ^b	0.28 ± 0.00 ^b	0.38 ± 0.24 ^b
Third sample	5.41 ± 0.00 ^b	5.68 ± 0.00 ^c	9.02 ± 0.00 ^c	6.70 ± 1.74 ^c
Fourth sample	4.12 ± 0.09 ^c	4.43 ± 0.48 ^d	0.39 ± 0.00 ^d	2.98 ± 1.96 ^d
Fifth sample	5.04 ± 0.05 ^d	6.72 ± 0.06 ^e	6.47 ± 0.09 ^e	6.07 ± 0.79 ^e
Overall particle size	3.19 ± 2.15 ^a	3.59 ± 2.69 ^b	3.42 ± 3.75 ^c	

* The determination of the mean value ± standard deviation in triplicate. There is a substantial ($p \leq 0.05$) difference in the mean values in the same column separated by distinct characters (different superscript letters). Before the first fermentation (first sample), after the first fermentation (second sample), before the second fermentation (third sample), after the second fermentation (fourth sample) and, lastly, the final product (fifth sample).

For the different sampling times and particle sizes, Table 7 shows the Umqombothi (+b*) yellowness values.

Table 7. The effect of particle size on the yellowness values of Umqombothi *.

Sampling Stages	Yellowness		
	Control	Coarse Powder	Fine Powder
First sample	2.72 ± 0.00 ^a	3.78 ± 0.00 ^a	4.42 ± 0.00 ^a
Second sample	2.44 ± 1.08 ^a	1.54 ± 0.00 ^b	2.22 ± 0.00 ^b
Third sample	19.52 ± 0.00 ^b	20.42 ± 0.00 ^c	24.18 ± 0.00 ^c
Fourth sample	19.73 ± 0.12 ^c	19.41 ± 0.64 ^d	1.34 ± 0.00 ^d
Fifth sample	18.74 ± 0.07 ^d	22.25 ± 0.06 ^e	21.97 ± 0.13 ^e
Overall particle size	12.63 ± 8.51 ^a	13.48 ± 9.22 ^b	10.83 ± 10.43 ^c

* The determination of the mean value ± standard deviation in triplicate. There is a substantial ($p \leq 0.05$) difference in the mean values in the same column separated by distinct characters (different superscript letters). Before the first fermentation (first sample), after the first fermentation (second sample), before the second fermentation (third sample), after the second fermentation (fourth sample) and, lastly, the final product (fifth sample).

Given that the values were positive (+b*), Umqombothi is thought to be in the (+b*) color space. This is to be anticipated because traditional beer is mainly made out of yellow maize [15,16].

The concentration of the solids influences the yellowness of Umqombothi. As a result, following sieving, the control particle size saw a substantial ($p < 0.05$) drop in the (+b*) and an increase ($p < 0.05$) in regard to the fine and coarse powder particle sizes. Umqombothi produced with a coarse particle size had a significantly greater yellowness at the fifth sampling stage than the fine powder and control particle sizes, respectively. The overall yellowness levels were substantially different ($p < 0.05$) for the control, coarse, and fine powder particle sizes, respectively, with the coarse particle size having higher values than those for the control and fine powder particle sizes.

Table 8 displays Umqombothi's chroma values from various particle sizes at various sample stages. The chroma of Umqombothi was positive during the Umqombothi production process. The way the solids in Umqombothi come together impacts how vivid it is, as seen in the effect of sieving. The overall chroma values, as affected by the particle sizes, exhibited substantial ($p < 0.05$) differences for the control, coarse, and fine powder particle sizes. Umqombothi produced with the coarse particle size had a more outstanding chroma than the control and fine powder particle sizes.

Table 8. The effect of particle size on the chroma values of Umqombothi *.

Sampling Stages	Chroma		
	Control	Coarse Powder	Fine Powder
First sample	2.83 ± 0.00 ^a	3.88 ± 0.00 ^a	4.52 ± 0.00 ^a
Second sample	2.52 ± 1.12 ^a	1.56 ± 0.00 ^b	2.23 ± 0.00 ^b
Third sample	20.25 ± 0.00 ^b	21.19 ± 0.00 ^c	25.80 ± 0.00 ^c
Fourth sample	20.16 ± 0.14 ^b	19.92 ± 0.50 ^d	1.39 ± 0.00 ^d
Fifth sample	19.41 ± 0.08 ^b	23.23 ± 0.08 ^e	22.63 ± 0.32 ^e
Overall particle size	13.03 ± 8.77 ^a	13.96 ± 9.59 ^b	11.31 ± 11.00 ^c

* The determination of the mean value ± standard deviation in triplicate. There is a substantial ($p \leq 0.05$) difference in the mean values in the same column separated by distinct characters (different superscript letters). Before the first fermentation (first sample), after the first fermentation (second sample), before the second fermentation (third sample), after the second fermentation (fourth sample) and, lastly, the final product (fifth sample).

The hue angle (h°) of Umqombothi made with different particle sizes at different sampling stages is presented in Table 9. The h° of Umqombothi remained positive during the production process for the control, coarse, and fine powder particle sizes.

Table 9. The impact of particle size on Umqombothi's hue angle level *.

Sampling Stages	Hue Angle (h°)		
	Control	Coarse Powder	Fine Powder
First sample	74.19 ± 0.00 ^{a,c}	76.68 ± 0.00 ^a	77.79 ± 0.00 ^a
Second sample	75.30 ± 0.56 ^{b,e}	81.87 ± 0.00 ^b	82.90 ± 0.00 ^b
Third sample	74.52 ± 0.00 ^{c,ae}	74.44 ± 0.00 ^c	69.55 ± 0.00 ^c
Fourth sample	78.19 ± 0.18 ^d	77.11 ± 1.79 ^a	73.94 ± 0.00 ^d
Fifth sample	74.93 ± 0.09 ^{e,cb}	73.19 ± 0.09 ^c	73.57 ± 0.14 ^e
Overall particle size	75.43 ± 1.50 ^a	76.66 ± 3.16 ^b	75.55 ± 4.66 ^c

* The determination of the mean value ± standard deviation in triplicate. There is a substantial ($p \leq 0.05$) difference in the mean values in the same column separated by distinct characters (different superscript letters). Before the first fermentation (first sample), after the first fermentation (second sample), before the second fermentation (third sample), after the second fermentation (fourth sample) and, lastly, the final product (fifth sample).

The overall h° values, affected by the particle size, exhibited substantial ($p < 0.05$) differences between the control, coarse, and fine powder particle sizes. Umqombothi produced with the coarse particle size had a greater hue angle than the fine powder and control particle sizes. The trend can be justified by what was reported by [37], stating that the temperature, oxidation of polyphenols, and grist material impacted the wort's color, as well as during the processing steps. The hue angles of the samples further suggested

that a yellowish color dominates the Umqombothi as they are close to a hue angle of 90° , representing pure yellowness.

The color distinction (ΔE) between Umqombothi made with distinct particle sizes ranged from 0.68 to 10.58. A color difference (ΔE) < 1 can be defined as a “not noticeable difference”, where the observer does not notice the difference. A color difference ($\Delta E = 1$) is a just noticeable difference (JND). A color difference between 4 and 8 is perceivable but accepted [38], implying that an observer notices the color difference and considers it acceptable.

The color difference between the Umqombothi samples with the control and coarse powder particle sizes was not noticeable, as shown by (ΔE) < 1 (0.68). The difference in color between the control and fine powder particle size was noticeable but acceptable because the color difference was 5.36. The difference in color between the coarse and fine powder was 10.58, which was unacceptable.

In the current study, using a spectrophotometer, we observed that Umqombothi is light, reddish, yellow in color.

3.3. Effect of Particle Size on Percentage Syneresis (%STS) of Umqombothi during the Production Process

In food science, syneresis is the process of extracting or expelling a liquid, such as water, from a gel. As a quality problem, syneresis is a big worry for product creators. It can detract from a food product’s appearance and is, first of all, ugly [19]. Table 10 displays the % syneresis of Umqombothi made with various particle sizes at various sample stages. There was a substantial ($p < 0.05$) difference in syneresis between all the sampling stages for the control, coarse, and fine powder particle sizes. There was a substantial ($p < 0.05$) decrease in syneresis from the first stage to the fifth stage. Cooking (third stage) resulted in a significant ($p < 0.05$) decrease, while sieving resulted in a significant ($p < 0.05$) increase in the syneresis of Umqombothi. This is due to the removal of the solids. The overall syneresis values exhibited substantial ($p < 0.05$) differences for the control, coarse, and fine powder particle sizes. The control particle size had the ($p < 0.05$) lowest syneresis value. This is due to the increased availability of starch during cooking, which affects food viscosity and describes the clarity of the finished product [3,9].

Table 10. The effect of particle size on the % STS value of Umqombothi *.

Sampling Stages	Syneresis (%)		
	Control	Coarse Powder	Fine Powder
First sample	95.81 $\pm$ 0.01 ^a	93.28 $\pm$ 0.01 ^a	91.36 $\pm$ 0.00 ^a
Second sample	89.29 $\pm$ 0.02 ^b	95.00 $\pm$ 0.01 ^b	90.78 $\pm$ 0.01 ^b
Third sample	0.00 $\pm$ 0.00 ^c	0.00 $\pm$ 0.00 ^c	0.00 $\pm$ 0.00 ^c
Fourth sample	28.29 $\pm$ 0.01 ^d	25.38 $\pm$ 1.73 ^d	33.45 $\pm$ 0.01 ^d
Fifth sample	42.57 $\pm$ 0.02 ^e	44.36 $\pm$ 0.02 ^e	46.19 $\pm$ 0.02 ^e
Overall particle size	51.19 $\pm$ 37.78 ^a	51.60 $\pm$ 38.80 ^b	52.36 $\pm$ 36.26 ^c

* The determination of the mean value $\pm$ standard deviation in triplicate. There is a substantial ($p \leq 0.05$) difference in the mean values in the same column separated by distinct characters (different superscript letters). Before the first fermentation (first sample), after the first fermentation (second sample), before the second fermentation (third sample), after the second fermentation (fourth sample) and, lastly, the final product (fifth sample).

3.4. Effect of Particle Size on the Viscosity of Umqombothi during the Production Process

3.4.1. The Effect of Particle Size on Umqombothi (Viscosity vs. Time)

The apparent viscosity vs. time in relation to the control, coarse, and fine powder particle sizes used in the production of Umqombothi, before and after fermentation, are presented in Figure 2. The second viscosity observed for the Umqombothi produced with the control particle size (A) was initially $400.0 \text{ MPa}\cdot\text{s}^{-1}$ at approximately 0 s and gradually increased to $800.0 \text{ MPa}\cdot\text{s}^{-1}$ over 15 s. The same effect was observed during the first stage, initially $-300.0 \text{ MPa}\cdot\text{s}^{-1}$ and gradually increased to $0.0 \text{ MPa}\cdot\text{s}^{-1}$ over 15 s (A).

Umqombothi made with the coarse particle size (fourth stage) was initially $100.0 \text{ MPa}\cdot\text{s}^{-1}$, which gradually increased to $200.0 \text{ MPa}\cdot\text{s}^{-1}$ over 15 s. During the first stage, it was initially $0.0 \text{ MPa}\cdot\text{s}^{-1}$ at approximately 0 s and gradually increased to $10.0 \text{ MPa}\cdot\text{s}^{-1}$ at 17 s, as indicated in (B). Umqombothi made with the fine powder particle size (fourth stage) was initially $150.0 \text{ MPa}\cdot\text{s}^{-1}$ at approximately 0 s and gradually increased to $220.0 \text{ MPa}\cdot\text{s}^{-1}$ at 17 s. The same effect was observed at first. It was initially $5.0 \text{ MPa}\cdot\text{s}^{-1}$ at approximately 0 s and gradually increased to $10.0 \text{ MPa}\cdot\text{s}^{-1}$ at 17 s (C). The control, coarse, and fine powder samples of Umqombothi had a thicker viscosity during the first stage than the second stage, respectively. The viscous properties related to the fermentation time of Umqombothi (before and after fermentation) showed an increasing effect when the shearing period was prolonged at a constant shear rate (500 s^{-1}). The observed tendency identifies Umqombothi as benefiting from shear thickening, since its viscosity increases when shear is applied rather than when it is absent or prolonged. Umqombothi is primarily prepared through the microbial metabolism of LAB, which can contribute to an increase in viscosity [20].

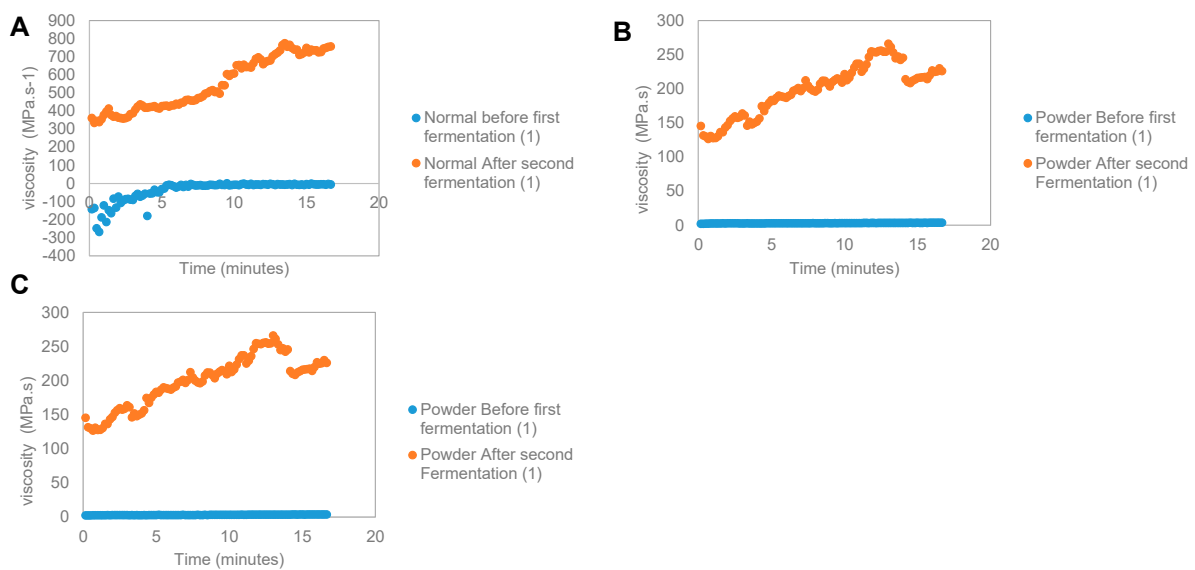


Figure 2. Apparent viscosity of (A) control particle, (B) coarse powder particle, and (C) fine powder particle sizes of Umqombothi as a function of time at a constant shear rate (500 s^{-1}). Before the first fermentation (BFF), after the second fermentation (ASF).

3.4.2. The Effect of Particle Size on Umqombothi (Viscosity vs. Temperature)

The apparent viscosity as a function of the fermentation temperature for the Umqombothi made with the control, coarse, and fine powder particle sizes (first and fourth stages) is presented in Figure 3. The control particle size during the fourth stage was initially $800.0 \text{ MPa}\cdot\text{s}^{-1}$ at approximately 0°C and gradually decreased to $200.0 \text{ MPa}\cdot\text{s}^{-1}$ at 25°C (Figure 3A). The same effect was observed during the first stage. It was initially $0.0 \text{ MPa}\cdot\text{s}^{-1}$ at approximately 0°C and gradually decreased to $-300.0 \text{ MPa}\cdot\text{s}^{-1}$ at 25°C . Umqombothi made with the coarse powder particle size (fourth stage) was initially $200.0 \text{ MPa}\cdot\text{s}^{-1}$ at approximately 0°C and gradually decreased to $100.0 \text{ MPa}\cdot\text{s}^{-1}$ at 25°C (Figure 3B). The same effect was observed during the first stage. It was initially $8.0 \text{ MPa}\cdot\text{s}^{-1}$ at approximately 3°C and gradually decreased to $4.0 \text{ MPa}\cdot\text{s}^{-1}$ at 25°C . Umqombothi made with the fine powder particle size (fourth stage) was initially $240.0 \text{ MPa}\cdot\text{s}^{-1}$ at approximately 3°C and gradually decreased to $100.0 \text{ MPa}\cdot\text{s}^{-1}$ at 25°C (Figure 3C). The same effect was observed during the first stage. It was initially $10.0 \text{ MPa}\cdot\text{s}^{-1}$ at approximately 4°C and gradually decreased to $5.0 \text{ MPa}\cdot\text{s}^{-1}$ at 25°C , as indicated.

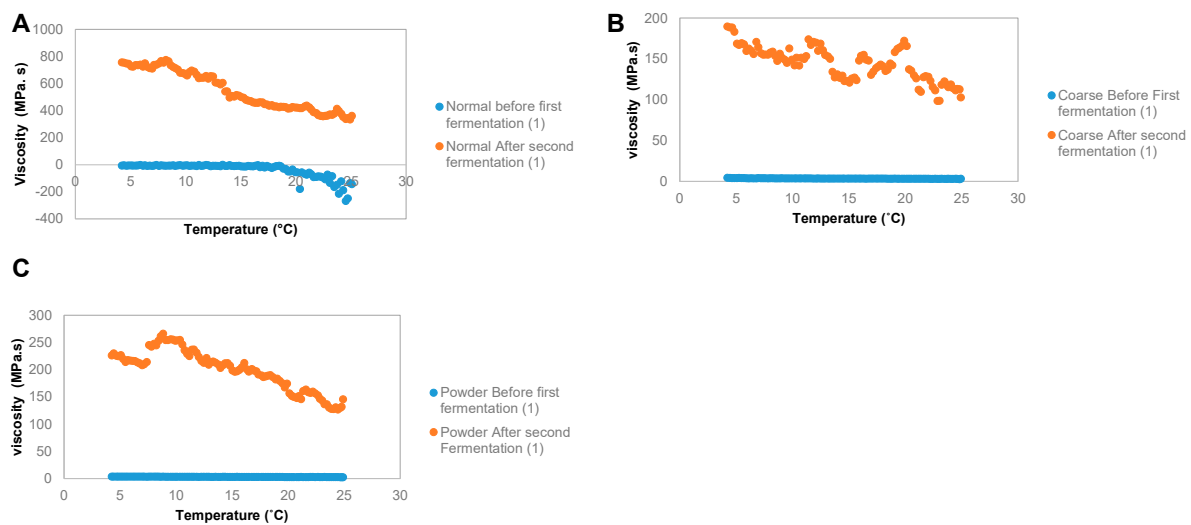


Figure 3. Apparent viscosity of Umqombothi made with the (A) control, (B) coarse powder, and (C) fine powder particle sizes, as a function of temperature at a constant shear rate (500 s⁻¹). Before the first fermentation (BFF), after the second fermentation (ASF).

The viscous properties of Umqombothi (first and fourth stage) showed a decreasing effect when the temperature was increased at a constant shear rate (500 s⁻¹). The above-observed trend reveals Umqombothi's shear-thinning pseudoplastic behavior, namely the absence of shear and temperature results in a higher viscosity and its application results in decreased viscosity. Before fermentation, the viscosity of all the beer samples (control, coarse, and fine powder particle sizes) in regard to the viscosity vs. temperature was higher than 200 MPa·s⁻¹. As much as the viscosity of all the beer samples decreased, they did not go below 100 MPa·s⁻¹. The viscosity of cereals decreases due to the conversion of starch by LAB into simpler sugars during fermentation. The pH, the type of microorganisms, and whether those microorganisms include amylase enzymes that hydrolyze starch into dextrin and sugars, all impact the beverage's viscosity [20]. The viscosity and nutritional value of the solution improved in various ways due to the breakdown of starch by LAB [20]. These findings concur with those of [39], who noted a reduction in viscosity following the fermentation of a conventional fermented beverage (Boza) at 20 °C. According to [39], heating reduces viscosity by decreasing the molecular entanglement and stabilizing the molecular structure of sugar and protein.

Finally, rheological information about Umqombothi may be helpful in the design and choice of machinery needed for industrial production. It is significant in this context to consider Umqombothi's pseudoplastic behavior concerning temperature, as indicated by the flow behavior index and consistency index values. The processing needs for the synthesis of Umqombothi may also depend on biochemical changes during fermentation, such as the pH and changes in water-soluble proteins.

3.5. Microbial Population in Umqombothi

The LAB counts during Umqombothi production for different particle sizes are displayed in Table 11. There was a significant ($p < 0.05$) difference between all the sampling stages for the coarse and fine powder particle sizes. The lactic acid bacteria (LAB) counts increased substantially ($p < 0.05$) after the fermentation stages for the control, coarse, and fine powder particle sizes. The most common microbe in sorghum during fermentation is lactic acid bacteria [9], with fewer reports and instances of fungus and yeast. The conditions that favor LAB growth are high in lipids, sugar, protein, vitamins, and nucleotides. [9]. This may account for their prevalence in the microflora of sorghum. Lactic acid bacteria were not detected in the third sampling stage for the control, coarse, and fine powder particle sizes. This could be due to the cooking temperature and time during mashing, which does

not favor LAB growth. The LAB increased ($p < 0.05$) between the first and fifth sampling stages for all three particle sizes. According to [9], these microbes are predatory rivals that hinder other microbes, by quickly consuming an abundance of glucose and building up acetic and lactic acid. The fifth sampling stage LAB values for the control, coarse, and powder particle sizes, respectively, are similar to the LAB values reported by [16] in regard to indigenous fermented maize. However, the results are higher than those reported by [17], of 4.94 log cfu/mL.

Table 11. The effect of particle size on lactic acid bacteria (LAB) count in Umqombothi *.

Sampling Stage	LAB (cfu/mL)		
	Control	Coarse Powder	Fine Powder
First sample	5.02 ± 0.08 ^a	4.99 ± 0.01 ^a	5.09 ± 0.02 ^a
Second sample	7.86 ± 0.02 ^b	7.93 ± 0.01 ^b	8.03 ± 0.07 ^b
Third sample	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c
Fourth sample	7.83 ± 0.03 ^b	7.63 ± 0.05 ^d	7.77 ± 0.04 ^d
Fifth sample	8.16 ± 0.11 ^e	7.11 ± 0.02 ^e	5.91 ± 0.09 ^e
Overall particle size	5.77 ± 3.21 ^a	5.53 ± 3.05 ^b	5.36 ± 3.00 ^c

* The determination of the mean value ± standard deviation in triplicate. There is a substantial ($p \leq 0.05$) difference in the mean values in the same column separated by distinct characters (different superscript letters). Before the first fermentation (first sample), after the first fermentation (second sample), before the second fermentation (third sample), after the second fermentation (fourth sample) and, lastly, the final product (fifth sample).

The overall LAB levels of 5.77, 5.53, and 5.36 log cfu/mL for the control, coarse, and powder particle sizes, respectively, are significantly lower than the LAB values of 8.56, 7.96, and 7.82 log cfu/mL reported by [16]. However, the levels are higher than those reported by [17] of 4.94 log cfu/mL. Cooking reduces the number of bacteria [40]. Heat treatment and fermentation improve the beverage's taste, odor, and digestibility. Starch is transformed into fermentable sugars, vitamins, and amino acids during heating. This process helps LAB and yeast grow, provide taste and smell, and maintain the sensory quality profile of Umqombothi beverages. They also extend the shelf life of the product by preventing bacteria, yeast, and mold growth, which can cause spoiling and poisoning [1,24]. The addition of red sorghum malt, before the second fermentation, increased the product's total microbial load. The microorganisms present during the manufacturing of Umqombothi beer have shown the same trend [41].

This is not surprising considering that LAB is among the most prevalent groups of several bacterial species that occur in the fermentation of sorghum. The TVC during the production of Umqombothi for the different particle sizes are displayed in Table 12. There was a significant ($p < 0.05$) difference in the TVC between all the sampling stages for the control, coarse, and fine powder particle sizes, respectively. There was a substantial increase ($p < 0.05$) in the TVC between the first and the fifth stages for all three particle sizes. The TVC was not detected for the control, coarse, and fine powder particle sizes in the third sampling stage. The increase in the TVC before the second fermentation stage is caused by the inclusion of malted sorghum and the starter culture. At the same time, the decrease in the TVC for the final product with the coarse and fine powder particle sizes was caused by the removal of solids during sieving. There was a significant ($p < 0.05$) difference in the overall TVC levels for the control, coarse, and fine powder particle sizes. According to [9,16], the results in the current study, regarding the fermentation and boiling of sorghum, as conducted in the production of Umqombothi, raises its nutritional value, while bringing the content of the anti-nutritional components down to a tolerable level. The substantial rise in the TVC in the finished product was caused by the raw material's elevated MC content, nutrition, and contaminated microorganisms [36]. If the affected malted sorghum had been added after the third heating step, the TVC in the finished product would have certainly increased dramatically. The overall TVC of 5.95, 5.58, and 5.38 log cfu/mL for the

control, coarse, and fine powder particle sizes, respectively, are lower than the TVC value of 8.66 cfu/mL reported for a sorghum beer final product by [17].

Table 12. The effect of particle size on the total viable count (TVC) of Umqombothi *.

Sampling Stage	Total Viable Count (cfu/mL)		
	Control	Coarse Powder	Fine Powder
First sample	5.62 ± 0.11 ^a	5.10 ± 0.03 ^a	5.37 ± 0.04 ^a
Second sample	7.95 ± 0.01 ^b	7.94 ± 0.02 ^b	8.04 ± 0.05 ^b
Third sample	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c
Fourth sample	7.79 ± 0.01 ^d	7.61 ± 0.03 ^d	7.71 ± 0.03 ^d
Fifth sample	8.39 ± 0.14 ^e	7.26 ± 0.04 ^e	5.81 ± 0.03 ^e
Overall particle size	5.95 ± 3.24 ^a	5.58 ± 3.07 ^b	5.38 ± 2.99 ^c

* The determination of the mean value ± standard deviation in triplicate. There is a substantial ($p \leq 0.05$) difference in the mean values in the same column separated by distinct characters (different superscript letters). Before the first fermentation (first sample), after the first fermentation (second sample), before the second fermentation (third sample), after the second fermentation (fourth sample) and, lastly, the final product (fifth sample).

Ref. [36] reported that the total aerobic count of the samples ranged from 5.7 to 10.8 log cfu/mL/ in the final product of Umqombothi. There was a significant ($p < 0.05$) difference in the yeast count between all the sampling stages for the control, coarse, and fine powder particle sizes. The yeast increased ($p < 0.05$) significantly between the first and fifth stage and decreased ($p < 0.05$) significantly between the fourth and fifth sampling stages for all three particle sizes (Table 13). First, a procedure known as “backslopping” is utilized to extract and repurpose beverage yeast to start the next fermentation batch [42]. The yeast reduction may be related to elimination of the solid particles during sieving. Alcoholic fermentation is one of the conventional processes that primarily defines the end product’s quality [43]. Yeast was not detected in the third sampling stage for the control, coarse, and fine powder particle sizes, which could be due to the cooking temperature and time.

Table 13. The effect of particle size on the yeast count in Umqombothi *.

Sampling Stage	Yeast (cfu/mL)		
	Control	Coarse Powder	Fine Powder
First sample	5.09 ± 0.01 ^a	5.09 ± 0.04 ^a	5.25 ± 0.01 ^a
Second sample	7.56 ± 0.03 ^b	7.14 ± 0.01 ^b	7.12 ± 0.02 ^b
Third sample	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c
Fourth sample	7.89 ± 0.05 ^d	7.79 ± 0.05 ^d	7.91 ± 0.06 ^d
Fifth sample	3.3 ± 0.12 ^e	4.64 ± 0.05 ^e	5.63 ± 0.03 ^e
Overall particle size	4.77 ± 3.01 ^a	4.93 ± 2.83 ^b	5.18 ± 2.86 ^c

* The determination of the mean value ± standard deviation in triplicate. There is a substantial ($p \leq 0.05$) difference in the mean values in the same column separated by distinct characters (different superscript letters). Before the first fermentation (first sample), after the first fermentation (second sample), before the second fermentation (third sample), after the second fermentation (fourth sample) and, lastly, the final product (fifth sample).

There was a significant ($p < 0.05$) difference in the overall yeast count between the control, coarse, and fine powder particle sizes. The fine powder particle size had the highest yeast value. The further along the fermentation process was, because of the existence of LAB, the lesser the ethanol, pH, and viscosity [20]. The overall yeast values of 4.77, 4.93, and 5.18 log cfu/mL for the control, coarse, and fine powder particle sizes, respectively, were lower than the levels reported for a Umqombothi final product by [16] of 6.52, 7.1, and 6.42 log cfu/mL, and a yeast value of 8.05 cfu/mL for Ivorian sorghum beer reported by [17].

Ref. [24] reported a yeast count of 2.3×10^7 cfu/mL for a Umqombothi final product. Several strains of *Saccharomyces cerevisiae* are utilized to create various beer styles. Although

the characteristics of each group vary, they all have characteristics of domestication, such as high flocculation, efficient sugar consumption, and a lack of unwanted flavors [44]. According to [27], the abundance of protein, sugar, vitamins, nucleotides, and lipids in the environment where yeast flourishes may account for their dominance in sorghum microflora. Because Umqombothi is nutritious, there was a significant rise in the yeast population following fermentation [3,16]. Natural fermentation results in increased microbial activity, a drop in pH, and the release of enzymes [40]. This results in the substrate breaking down and an improvement to the nutritional quality, which promotes yeast growth [45].

The yeast counts obtained in this study confirm that yeast is one of the most dominant microorganisms in Umqombothi. The yeast counts also demonstrate the effect of particle size on Umqombothi. It is common to undervalue a yeast strain's impact on a product's quality. However, the specific strain will significantly impact the beer's character as a raw material [44]. Depending on the type of yeast used to create a specific style, brewing yeast affects the taste and character of beer. Traditional fermented drinks may be a rich source of yeast strains for the brewing industry. Many ancient methods of producing fermented beer and other beverages include the spontaneous initiation of a mixture of local yeast strains, in addition to *S. cerevisiae*. Yeast converts fermentable carbohydrates into ethanol, creating many active flavor chemicals [44].

The number of molds, used in the manufacturing of Umqombothi, is displayed for each particle size in Table 14.

Table 14. The effect of particle size on the mold count of Umqombothi *.

Sampling Stage	Mold (cfu/mL)		
	Control	Coarse Powder	Fine Powder
First sample	4.06 ± 0.10 ^a	4.29 ± 0.09 ^a	4.08 ± 0.04 ^a
Second sample	4.39 ± 3.81 ^b	1.67 ± 2.89 ^b	3.33 ± 2.89 ^b
Third sample	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c
Fourth sample	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c
Fifth sample	2.27 ± 0.22 ^d	3.30 ± 0.30 ^d	0.00 ± 0.00 ^c
Overall particle size	2.14 ± 2.43 ^a	1.85 ± 2.10 ^a	1.48 ± 2.19 ^a

* The determination is the mean value ± standard deviation in triplicate. There is a substantial ($p \leq 0.05$) difference in the mean values in the same column separated by distinct characters (different superscript letters). Before the first fermentation (first sample), after the first fermentation (second sample), before the second fermentation (third sample), after the second fermentation (fourth sample) and, lastly, the final product (fifth sample).

No molds were detectable in the third to fourth sampling stages for the control, coarse, and fine powder particle sizes; it could be that the fungi and its spores were destroyed during cooking. The mold counts dropped ($p < 0.05$) considerably to non-detected for all three particle sizes between the first and fourth sample stages. The pH significantly ($p < 0.05$) decreased from the first to fifth stages see Table 1, as the LAB count increased from the first to the fifth stage, resulting in a significant ($p < 0.05$) decrease in the mold count to non-detected for the control, coarse, and fine powder particle sizes.

There was a significant ($p < 0.05$) difference in the overall mold count between the control, coarse, and fine powder particle sizes. The control particle size exhibited the highest mold counts ($p < 0.05$). An unfavorable fermentation temperature and pH environment reduced the molds during the Umqombothi production process. This situation accounts for the noteworthy ($p < 0.05$) decline in mold colonies. The mold counts were also impacted by the elimination of solid particles and the pH drop during the fifth sample step. The notable mold reduction in the Umqombothi production process before the second fermentation sampling stage aligns with the findings in [1], while producing a white beverage known as “mpedli”. Umqombothi breaks down quickly when exposed to mold, which may be one of the reasons it only lasts for two to three days [24,46]. The total coliform count during Umqombothi production for the different particle sizes is displayed in Table 15. There was no significant difference between the fourth and fifth sampling stages for the

control particle size during the Umqombothi production process. This could be due to the low pH at the fourth and fifth sampling stages (3.54–3.45). The total coliform count decreased significantly ($p < 0.05$) during the first, second, and fourth sampling stages for the control, coarse, and fine powder particle sizes, indicating that the conditions during the first and second fermentation stages were not ideal for coliforms to proliferate. No detectable coliforms were present after cooking (third sampling stage), and they remained absent during the fourth sampling stage in regard to the control particle size.

Table 15. The effect of particle sizes on the total coliform count of Umqombothi *.

Sampling Stages	Total Coliforms (cfu/mL)		
	Control	Coarse Powder	Fine Powder
First sample	4.82 ± 0.04^a	4.86 ± 0.06^a	5.16 ± 0.05^a
Second sample	4.63 ± 0.03^b	3.52 ± 0.07^b	3.79 ± 0.06^b
Third sample	0.00 ± 0.00^c	0.00 ± 0.00^c	0.00 ± 0.00^c
Fourth sample	0.00 ± 0.00^c	3.79 ± 0.02^d	3.74 ± 0.03^d
Fifth sample	0.00 ± 0.00^c	0.00 ± 0.00^c	0.00 ± 0.00^c
Overall particle size	1.89 ± 2.39^a	2.43 ± 2.11^b	2.54 ± 2.21^c

* The determination is the mean value $\pm$ standard deviation in triplicate. There is a substantial ($p \leq 0.05$) difference in the mean values in the same column separated by distinct characters (different superscript letters). Before the first fermentation (first sample), after the first fermentation (second sample), before the second fermentation (third sample), after the second fermentation (fourth sample) and, lastly, the final product (fifth sample).

The overall coliform count between the control, coarse, and fine powder particle sizes differed significantly ($p < 0.05$). Coliforms are facultative, anaerobic, aerobic bacteria that digest lactose in an acidic, gassy environment [42,43]. They require temperatures between 35 and 37 °C to grow [47,48]. Since water was one of the main elements used to make Umqombothi, a total coliform product analysis was necessary [49]. The results indicated that the heating and fermentation stages were important in controlling coliform bacteria during production [50].

3.6. Principal Components in Umqombothi during the Production Process

Principal component analysis was applied to the data to simplify the interpretation of the data. As shown in Figure 4, the PCA reduced the measured variables of the Umqombothi production process to three main components (F1, F2, and F3). F1 and F2 (Figure 4A) explained 67.4%, F1 and F3 (Figure 4B) explained 65.6%, and F2 and F3 (Figure 4C) explained 59% of the total data variance, respectively, for the Umqombothi production process involving different particle sizes. Figure 4 was created to explore the positive relationship between the parameters studied during the sampling stages. As shown in Figure 4, the variances could be separated into two groups. The first group is composed of color and the second group comprises microbes. Figure 4A shows that a positive correlation was found between the F1 (chroma, yellowness, redness, alcohol, gravity) and F2 components (mold, pH, LAB, yeast syneresis, TPC).

There was also a negative correlation between these compounds. Figure 4B, shows a positive correlation between the F1 (yellowness, redness, chroma, Brix) and F3 components (TPC, LAB, yeast). However, there was a negative correlation between these compounds and sampling stages before the second fermentation, “after cooking”, after the first and after the second fermentation. Figure 4C, shows a positive correlation between the F2 (LAB, TPC, yeast, hue angle) and F3 components (chroma, yellowness, redness, Brix). No variance was strongly correlated to before the second fermentation (after cooking) sampling stage. As shown in (Figure 4D), the measured variable of Umqombothi with different particle sizes was reduced to two main components (F1 and F2) by the PCA, namely F1 and F2, explaining 100% of the total data variance. A positive correlation was found between the F1 component (syneresis, yeast, gravity, Brix, coliforms), strongly correlated with the fine powder particle size, and the F2 component (TPC, LAB, mold, pH).

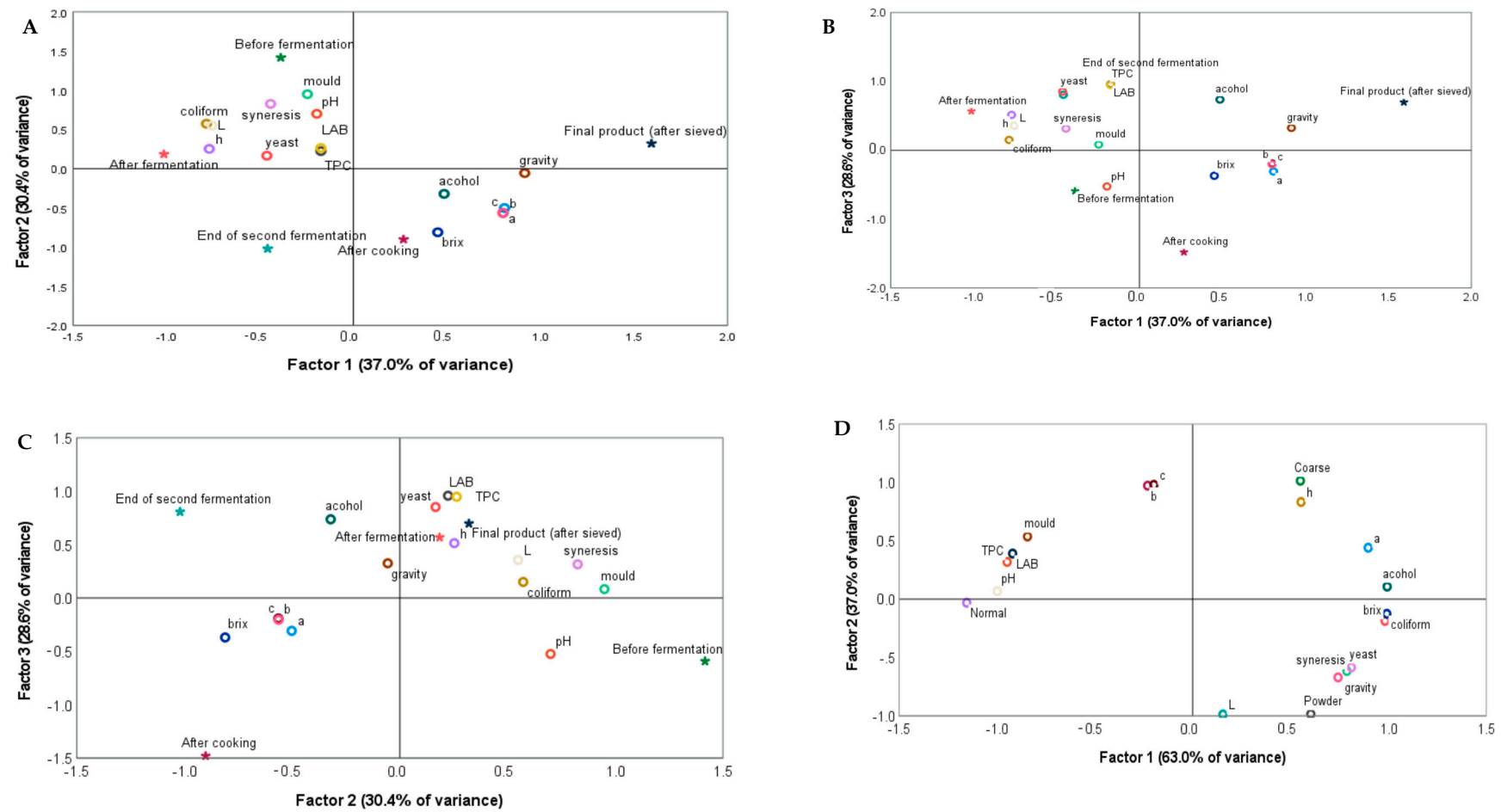


Figure 4. Principal component analysis (PCA) of the three different particle sizes used during the Umqombothi production process. A correlation was studied between (A) (F1–F2), (B) (F1–F3), (C) (F2–F3) and (D) (F1–F2).

3.7. Sensory Characteristics of Umqombothi

Table 16 displays the findings of the sensory assessment of Umqombothi made with the three distinct particle sizes. There was no significant difference regarding the appearance and texture between the Umqombothi prepared from the control, coarse, and fine powder particle sizes, as these aspects achieved a higher value of 3, namely neither like or dislike. Umqombothi made from the control particles had a value of 4, namely like moderately higher rating for color, aroma, taste, and overall acceptability than the rating of 3, namely neither like or dislike, for the coarse and fine powder Umqombothi (final product). The usual component particle sizes influenced the beer's overall taste profile, although it was relatively similar since the control particle size creates less alcohol than the coarse and fine powder particle sizes. Umqombothi produced with control particle sizes received better or higher scores in the consumer sensory evaluation, but the scores were not significantly different.

Table 16. The sensory evaluation of Umqombothi (final product) made from different particle sizes *.

Umqombothi Made from Different Particle Sizes			
Sensory Attributes	Control	Coarse Powder	Fine Powder
Appearance	3.82 ± 0.89 ^a	3.76 ± 1.21 ^a	3.66 ± 1.19 ^a
Color	4.1 ± 0.76 ^a	3.8 ± 1.16 ^a	3.72 ± 1.11 ^a
Aroma	4.02 ± 0.96 ^a	3.80 ± 0.81 ^a	3.66 ± 0.10 ^a
Taste	4.14 ± 0.97 ^a	3.86 ± 1.13 ^{ab}	3.62 ± 1.18 ^{bc}
Texture	3.82 ± 1.03 ^a	3.98 ± 1.14 ^a	3.54 ± 1.19 ^a
Overall acceptability	4.02 ± 0.94 ^a	3.94 ± 1.06 ^a	3.74 ± 1.03 ^a

* The determination is the mean value ± standard deviation in triplicate. There is a substantial ($p \leq 0.05$) difference in the mean values in the same column separated by distinct characters (different superscript letters). Before the first fermentation (first sample), after the first fermentation (second sample), before the second fermentation (third sample), after the second fermentation (fourth sample) and, lastly, the final product (fifth sample).

The second sensory evaluation study was conducted by comparing the sensory attributes of Umqombothi produced in a laboratory with 30–30 °C fermentation temperatures to traditional Umqombothi produced using coarse particle sizes in the township of Mbekweni (Langabuya) and New-Rest (Ezimbacwini) in Cape Town and Paarl in the Drakenstein Municipality (Figure 5, Table 17 present the overall percentage (%) acceptability according to the beverage ratings for the Umqombothi produced in the laboratory and the townships of Langabuya and Ezimbacwini). The panel comprised of approximately 34.7% males, 65.3% females, 85.7% black people, 12.2% people of mixed ancestry, and 2% white people. Additionally, 39.6% were CPUT staff members, 60.4% were students, and 10% were international students. Sixty-nine per cent (69%) were under 30 years old, 24.5% were between 30 and 39 years old, and 14.3% were over 40 years old.

The appearance distributions for the laboratory at Langabuya and Ezimbacwini Umqombothi are symmetrical, with the laboratory skewing upwards and Ezimbacwini skewing downwards. At the higher end, Ezimbacwini Umqombothi is more consistent. The median consumer panelist for all three Umqombothi samples was around 4, while the interquartile for the laboratory was around 3, better than Langabuya and Ezimbacwini. The middle 50% spread was wider in the laboratory at Umqombothi. Ezimbacwini Umqombothi had a range of 3, while the laboratory at Langabuya Umqombothi had a range of 4. Umqombothi laboratory had the most significant overall spread. The laboratory and Ezimbacwini Umqombothi have relatively symmetrical color distributions, whereas Langabuya Umqombothi was skewed upwards. Langabuya Umqombothi had a higher consistency at the upper end, whereas the laboratory and Ezimbacwini Umqombothi have a more significant color variation but have the same four medians as Langabuya Umqombothi. The interquartile for the laboratory and Ezimbacwini Umqombothi was 2, which was higher than the interquartile for Langabuya Umqombothi. Therefore, the middle 50% spread was large in the laboratory and Ezimbacwini Umqombothi. Ezimbacwini and the

laboratory had a color range of 4, while Langabuya Umqombothi had a color range 2. Overall, Ezimbacwini Umqombothi and the laboratory had a large spread.

The aroma median for the laboratory at Umqombothi was 3, while the aroma median for Langabuya and Ezimbacwini Umqombothi was 4. The laboratory and Langabuya Umqombothi had an aroma interquartile of 2, while Ezimbacwini had an aroma of 1. As a result, the dispersion of the middle 50% was better in the laboratory and Langabuya Umqombothi. The scent range of the laboratory and Langabuya Umqombothi was four, based on the complete aroma dataset. As a result, the laboratory and Langabuya Umqombothi had the greatest spread. Ezimbacwini Umqombothi was constantly in the middle, with a low outlier. The laboratory, Langabuya, and Ezimbacwini Umqombothi samples all had a taste median of 4 and a flavor range of 4. Ezimbacwini had a taste interquartile of 2, while the laboratory and Langabuya had a taste interquartile of 3. The middle 50% dispersion was more comprehensive in the laboratory and Langabuya Umqombothi. Overall, the laboratory and Langabuya have a more comprehensive range.

The texture median in all three Umqombothi samples was 4, the texture range was 4, and the texture interquartile was 2. According to the dataset, the laboratory, Langabuya, and Ezimbacwini Umqombothi all had the same spread, the same spread in the middle 50%, and the same overall spread. The skew in the laboratory and Ezimbacwini was downward. Langabuya and Ezimbacwini Umqombothi are consistent in the middle, while laboratory and Ezimbacwini Umqombothi have a more significant variation in the overall acceptance. The total acceptability median for the Umqombothi samples from the laboratory, Langabuya, and Ezimbacwini was 4. The overall acceptance range for the laboratory and Ezimbacwini Umqombothi was 3, whereas Langabuya Umqombothi was 4. The laboratory and Ezimbacwini Umqombothi had the most incredible spread throughout the dataset. Langabuya Umqombothi had a wider middle 50% spread of 2 than the laboratory and Ezimbacwini Umqombothi. Langabuya had the broadest range of overall acceptability. Compared to Langabuya and Ezimbacwini Umqombothi, the laboratory at Umqombothi exhibited the most variety in terms of all the sensory qualities and the highest median, range, and spread in the middle 50%.

The variations in Umqombothi could be attributed to the varying amounts of ingredients used, the cooking temperature and time, the fermentation temperature and time, the hygienic utensils used, and the levels of hydrolytic enzymes in the various cereal malts. The tannin levels in the various cereals may have also influenced the acceptability of the Umqombothi, due to the astringency associated with high tannin levels [26,51].

Table 17. Demographics of the panelists.

Item		Frequency (%)
Gender	Male	34.7
	Female	65.3
Race	Black people	85.7
	Mixed ancestry	12.2
	White people	2
Staff or student	Staff	39.6
	Student	60.4
International	Yes	10
	No	90
Age	<20–29	69
	30–39	24.5
	40 and above	14.3

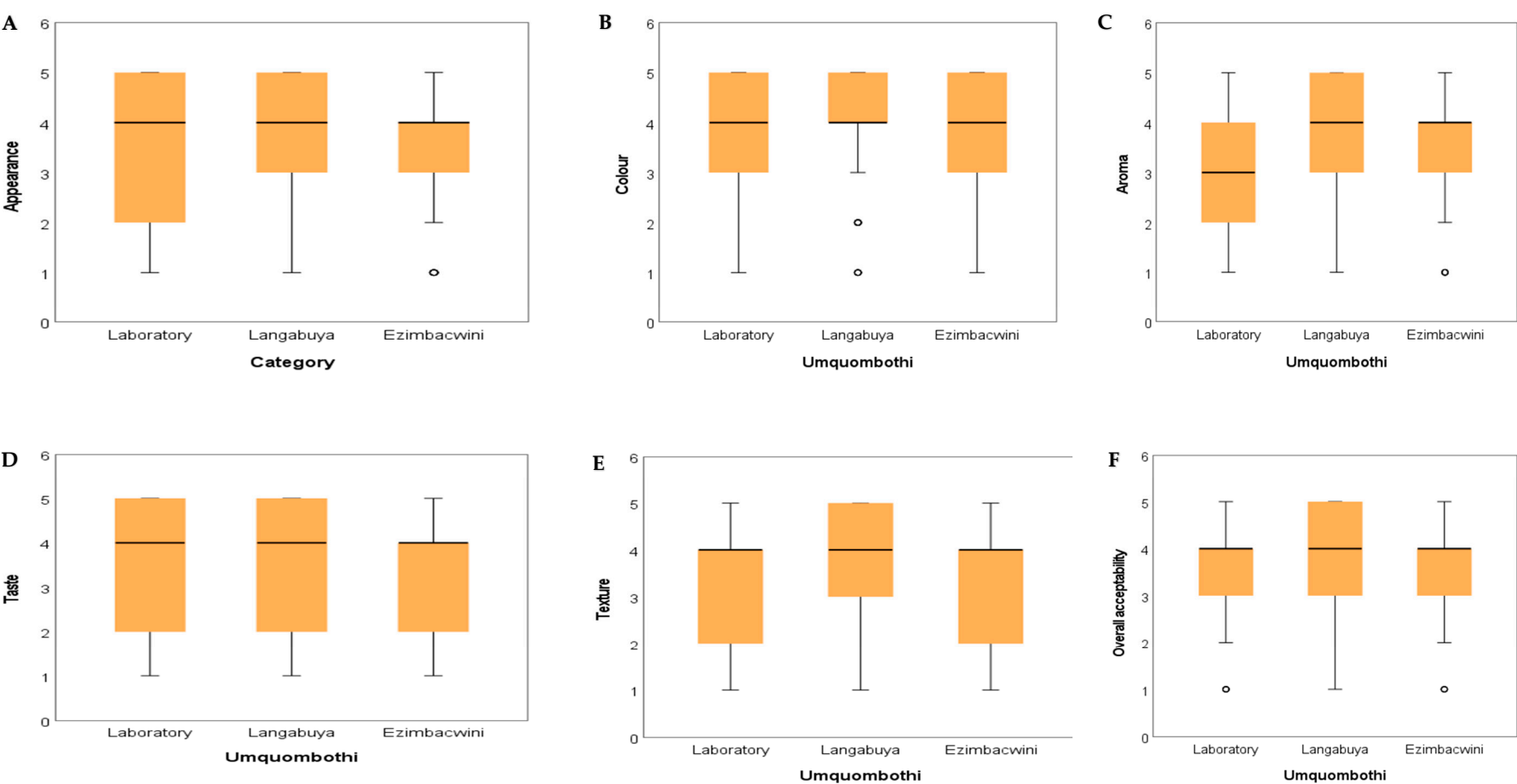


Figure 5. The distribution of sensory attributes: (A) appearance, (B) color, (C) aroma, (D) taste, (E) texture, and (F) overall acceptability.

4. Conclusions

In conclusion, the present study shows that different particle sizes of Umqombothi ingredients (sorghum and maize malt) affect the production process and the physicochemical, microbiological, and sensory characteristics of the final Umqombothi alcoholic beverage product. This is one aspect of the knowledge that is needed to produce Umqombothi commercially. A coarse powder particle size produced Umqombothi with a better chemical quality (lower pH and higher alcohol content) than the control and fine powder particle sizes. The control displayed better syneresis qualities and higher values during the sensory evaluation, as many of the values were not statistically different. The particle size further elaborates that the overall quality of Umqombothi produced under control fermentation temperatures can be improved without changing the formulation of the Umqombothi beverage and it is comparable in regard to its sensory qualities to the traditional Umqombothi produced with uncontrolled fermentation temperatures around the township of Mbekweni. As the production of this beverage has essential implications for the country's food system and economy, further studies are required to find the compounds that mainly determine the acceptability or rejection of the product, resulting in the development of a better preservation strategy using inoculation with pure yeast and LAB culture and improving the malt quality of sorghum and maize malt. Umqombothi is one of the traditional "beer style" beverages in South African, the understanding of which is very important to be able to produce it on a global scale, not only for traditional ceremonies but also to present it in the Beer Judge certification program.

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Data Availability Statement: The data generated in this study are the intellectual property of CPUT and are deposited in the CPUT library repository. The data are available upon request.

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References

1. Bayoï, J.R.; Etoa, F.X. Physicochemical changes occurring during long-time fermentation of the indigenous alcoholic sorghum-based beverages brewed in northern Cameroon. *Beverages* **2021**, *7*, 39. [\[CrossRef\]](#)
2. Bvochora, J.M.; Zvauya, R. Biochemical changes occurring during the application of high gravity fermentation technology to the brewing of Zimbabwean traditional opaque beer. *Process Biochem.* **2001**, *37*, 365–370. [\[CrossRef\]](#)
3. Schaepdrijver, D. Native African beers. In *Brewing*; Briggs, D.E., Boulton, C.A., Brookes, P.A., Stevens, R., Eds.; Elsevier: New York, NY, USA, 2004; pp. 589–605. [\[CrossRef\]](#)
4. Lyumugabe, F.; Gros, J.; Nzungize, J.; Bajyana, E.; Thonart, P. Characteristics of African traditional beers brewed with sorghum malt: A review. *Biotechnol. Agron. Soc. Environ.* **2012**, *16*, 509–530.
5. Tétédédé, C.; Konfo, R.; Chabi, N.W.; Dahouenon-ahoussi, E.; Cakpo-chichi, M.; Soumanou, M.M.; Coco, D.; Sohounhloué, K. Improvement of african traditional sorghum beers quality and potential applications of plants extracts for their stabilization: A review. *J. Microbiol. Biotechnol. Food Sci.* **2015**, *5*, 190–196. [\[CrossRef\]](#)

6. Usai, T.; Nyamunda, B.C.; Mutohoda, B. Malt Quality Parameters of Finger Millet for Brewing Commercial Opaque Beer. *Int. J. Sci. Res.* **2013**, *2*, 146–149.
7. Simatende, P.; Gadaga, T.H.; Nkambule, S.J. Methods of preparation of Swazi traditional fermented foods. *J. Ethn. Foods* **2015**, *2*, 119–125. [[CrossRef](#)]
8. Cason, E.D.; Mahlomaholo, B.J.; Taole, M.M.; Abong, G.O.; Vermeulen, J.G.; de Smidt, O.; Vermeulen, M.; Steyn, L.; Valverde, A.; Viljoen, B. Bacterial and Fungal Dynamics During the Fermentation Process of Sesotho, a Traditional Beer of Southern Africa. *Front. Microbiol.* **2020**, *11*, 1451. [[CrossRef](#)]
9. Hlangwani, E.; Adebiyi, J.A.; Doorsamy, W.; Adebo, O.A. Processing, Characteristics and Composition of Umqombothi (a South African Traditional Beer) Edwin: Review. *Processes* **2020**, *8*, 1451. [[CrossRef](#)]
10. Mousia, Z.; Balkin, R.C.; Pandiella, S.S.; Webb, C. The effect of milling parameters on starch hydrolysis of milled malt in the brewing process. *Process Biochem.* **2004**, *39*, 2213–2219. [[CrossRef](#)]
11. Linko, M.; Haikara, A.; Ritala, A.; Penttilä, M. Recent advances in the malting and brewing industry¹Based on a lecture held at the symposium 'Biotechnology in advanced food and feed processing', at the 8th European Congress on Biotechnology (ECB8) in Budapest, Hungary, August 1997.1. *J. Biotechnol.* **1998**, *65*, 85–98. [[CrossRef](#)]
12. Niemi, P.; Faulds, C.B.; Sibakov, J.; Holopainen, U.; Poutanen, K.; Buchert, J. Effect of a milling pre-treatment on the enzymatic hydrolysis of carbohydrates in brewer's spent grain. *Bioresour. Technol.* **2012**, *116*, 155–160. [[CrossRef](#)] [[PubMed](#)]
13. Langenaeken, N.A.; De Schepper, C.F.; De Schutter, D.P.; Courtin, C.M. Different gelatinization characteristics of small and large barley starch granules impact their enzymatic hydrolysis and sugar production during mashing. *Food Chem.* **2019**, *295*, 138–146. [[CrossRef](#)] [[PubMed](#)]
14. Bentley, I. Enzymes in brewing. *Brew. Distill.* **2006**, *2*, 34–37. [[CrossRef](#)]
15. Rumler, R.; Bender, D.; Speranza, S.; Frauenlob, J.; Gamper, L.; Hoek, J.; Jäger, H.; Schönlechner, R. Chemical and physical characterization of sorghum milling fractions and sorghum whole meal flours obtained via stone or roller milling. *Foods* **2021**, *10*, 870. [[CrossRef](#)] [[PubMed](#)]
16. Katongole, J.N. The Microbial Succession in Indigenous Fermented Maize Products. Ph.D. Thesis, University of the Free State, Bloemfontein, South Africa, February 2008; pp. 1–162.
17. Attchelouwa, C.K.; Aka-Gbézo, S.; N'guessan, F.K.; Kouakou, C.A.; Djè, M.K. Biochemical and Microbiological Changes during the Ivorian Sorghum Beer Deterioration at Different Storage Temperatures. *Beverages* **2017**, *3*, 43. [[CrossRef](#)]
18. Hardy, Z.; Jideani, V.A. Functional characteristics and microbiological viability of foam-mat dried Bambara groundnut (*Vigna subterranea*) yogurt from reconstituted Bambara groundnut milk powder. *Food Sci. Nutr.* **2020**, *8*, 5238–5248. [[CrossRef](#)] [[PubMed](#)]
19. Oyeyinka, S.A.; Singh, S.; Ma, Y.; Amonsou, E.O. Influence of high-pressure homogenization on the physicochemical properties of Bambara starch complexed with lysophosphatidylcholine. *LWT Food Sci. Technol.* **2016**, *74*, 120–127. [[CrossRef](#)]
20. Jideani, V.A.; Ratau, M.A.; Okudoh, V.I. Non-alcoholic pearl millet beverage innovation with own bioburden: *Leuconostoc mesenteroides*, *pediococcus pentosaceus* and *enterococcus gallinarum*. *Foods* **2021**, *10*, 1447. [[CrossRef](#)]
21. SANAS 4833:2007; Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Microorganisms—Colony-Count Technique at 30 °C. SANS: Cape Town, Africa, 2007.
22. ISO 4833:2003; Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Microorganisms—Colony-Count Technique at 30 Degrees C. ISO: Genewa, Switzerland, 2003.
23. Salmerón, I.; Thomas, K.; Pandiella, S.S. Effect of potentially probiotic lactic acid bacteria on the physicochemical composition and acceptance of fermented cereal beverages. *J. Funct. Foods* **2015**, *15*, 106–115. [[CrossRef](#)]
24. Adekoya, I.; Obadina, A.; Adaku, C.C.; De Boevre, M.; Okoth, S.; De Saeger, S.; Njobeh, P. Mycobiota and co-occurrence of mycotoxins in South African maize-based opaque beer. *Int. J. Food Microbiol.* **2018**, *270*, 22–30. [[CrossRef](#)]
25. Rogers, C.M.; Veatch, D.; Covey, A.; Staton, C.; Bochman, M.L. Terminal acidic shock inhibits sour beer bottle conditioning by *Saccharomyces cerevisiae*. *Food Microbiol.* **2016**, *57*, 151–158. [[CrossRef](#)] [[PubMed](#)]
26. Atter, A.; Obiri-danso, K.; Anyebuno, G.A.; Amoa-awua, W.K. Effect of Fermentation on the Chemical Quality of Burukutu a. *Ann. Food Sci. Technol.* **2017**, *18*, 349–354.
27. Hlangwani, E.; Adebiyi, J.A.; Adebo, O.A. Nutritional compositions of optimally processed umqombothi (A South African indigenous beer). *Fermentation* **2021**, *7*, 255. [[CrossRef](#)]
28. Hlangwani, E.; Doorsamy, W.; Adebiyi, J.A.; Fajimi, L.I.; Adebo, O.A. A modeling method for the development of a bioprocess to optimally produce umqombothi (a South African traditional beer). *Sci. Rep.* **2021**, *11*, 20626. [[CrossRef](#)]
29. Kutyaupipo, J.; Parawira, W.; Tinofa, S.; Kudita, I.; Ndengu, C. Investigation of shelf-life extension of sorghum beer (Chibuku) by removing the second conversion of malt. *Int. J. Food Microbiol.* **2009**, *129*, 271–276. [[CrossRef](#)]
30. Almonacid, S.F.; Nájera, A.L.; Young, M.E.; Simpson, R.J.; Acevedo, C.A. A Comparative Study of Stout Beer Batch Fermentation Using Free and Microencapsulated Yeasts. *Food Bioprocess Technol.* **2012**, *5*, 750–758. [[CrossRef](#)]
31. Entwistle, J.; Garrett, J.; Elahi, S.; Roper, P. Investigations into the effect of selected storage conditions on the alcohol content and original gravity of beers samples. *Food Control* **2008**, *19*, 461–464. [[CrossRef](#)]
32. Spedding, G. Alcohol and Its Measurement. In *Brewing Materials and Processes: A Practical Approach to Beer Excellence*; Elsevier Inc.: Lexington, KY, USA, 2016; pp. 123–149. [[CrossRef](#)]
33. Vanderhaegen, B.; Delvaux, F.; Daenen, L.; Verachtert, H.; Delvaux, F.R. Aging characteristics of different beer types. *Food Chem.* **2007**, *103*, 404–412. [[CrossRef](#)]

34. Dusabe, A.; Habamenshi, I.; Munyeshyashaka, E.; Niyonzima, F.N. Physicochemical and microbiological properties of umuranyoni, ubuki and ikigaga produced in Cyanika sector. *INES Sci. J.* **2021**, *1*, 18–24. [\[CrossRef\]](#)
35. Puligundla, P.; Smogrovicova, D.; Mok, C. Recent innovations in the production of selected specialty (non-traditional) beers. *Folia Microbiol.* **2021**, *66*, 525–541. [\[CrossRef\]](#)
36. Adekoya, I.; Obadina, A.; Olorunfemi, M.; Akande, O.; Landschoot, S.; De Saeger, S.; Njobeh, P. Occurrence of bacteria and endotoxins in fermented foods and beverages from Nigeria and South Africa. *Int. J. Food Microbiol.* **2019**, *305*, 108251. [\[CrossRef\]](#)
37. Pahl, R.; Meyer, B.; Biurrun, R. Wort and Wort Quality Parameters. In *Brewing Materials and Processes: A Practical Approach to Beer Excellence*; Kellershohn, J., Ed.; Elsevier Inc.: Berlin, Germany, 2016; pp. 113–121. [\[CrossRef\]](#)
38. Murevanhema, Y.Y.; Jideani, V.A. Production and Characterization of Milk Produced from Bambara Groundnut (*Vigna subterranea*) Varieties. *J. Food Process. Preserv.* **2015**, *39*, 1485–1498. [\[CrossRef\]](#)
39. Hayta, M.; Alpaslan, M.; Köse, E. The effect of fermentation on viscosity and protein solubility of Boza, a traditional cereal-based fermented Turkish beverage. *Eur. Food Res. Technol.* **2001**, *213*, 335–337. [\[CrossRef\]](#)
40. Russel, I. *Understanding Yeast Fundamentals*, 4th ed.; Nottingham University Press: Nottingham, UK, 2003; Available online: https://edisciplinas.usp.br/pluginfile.php/2993570/mod_resource/content/1/The_Alcohol_Textbook-4Ed.pdf (accessed on 15 February 2019).
41. Bayoï, J.R.; Etoa, F.-X. Assessment of Microbiological Quality and Safety during the Processing of Traditional Beers made from Sorghum in the “Mandara” Mountains of the Far-North Region of Cameroon. *Eur. J. Biol. Biotechnol.* **2021**, *2*, 74–84. [\[CrossRef\]](#)
42. Gallone, B.; Mertens, S.; Gordon, J.L.; Maere, S.; Verstrepen, K.J.; Steensels, J. Origins, evolution, domestication and diversity of *Saccharomyces* beer yeasts. *Curr. Opin. Biotechnol.* **2018**, *49*, 148–155. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Coulibaly, W.H.; Cot, M.; N’guessan, K.F.; Coulibaly, I.; Rigou, P.; Djè, K.M. Ethanol effect on yeast strains isolated from tchapalo, a traditional sorghum beer from Côte d’Ivoire. *Int. Food Res. J.* **2018**, *25*, 612–619.
44. Cubillos, F.A.; Gibson, B.; Grijalva-Vallejos, N.; Krogerus, K.; Nikulin, J. Bioprospecting for brewers: Exploiting natural diversity for naturally diverse beers. *Yeast* **2019**, *36*, 383–398. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Mensah, P. Fermentation—The key to food safety assurance in Africa? *Food Control* **1997**, *8*, 271–278. [\[CrossRef\]](#)
46. Blandino, A.; Al-aseeri, M.E.; Pandiella, S.S.; Cantero, D.; Webb, C. Cereal-based fermented foods and beverages. *Food Res. Int.* **2003**, *36*, 527–543. [\[CrossRef\]](#)
47. Eneje, L.O.; Obiekezie, S.O.; Aloh, C.U.; Agu, R.C. Effect of milling and mashing procedures on millet (*Pennisetum maiwa*) malt wort properties. *Process Biochem.* **2001**, *36*, 723–727. [\[CrossRef\]](#)
48. Hill, A.E. Microbiology. In *Brewing Materials and Processes: A Practical Approach to Beer Excellence*; Hill, A.E., Ed.; Elsevier Inc.: Endinburgh, SCT, UK, 2016; pp. 291–315. [\[CrossRef\]](#)
49. Eumann, M.; Schaeberle, C. Water. In *Brewing Materials and Processes: A Practical Approach to Beer Excellence*; Elsevier Inc.: Gärtringen, Germany, 2016; pp. 97–111. [\[CrossRef\]](#)
50. Sawadogo-Lingani, H.; Owusu-Kwarteng, J.; Glover, R.; Diawara, B.; Jakobsen, M.; Jespersen, L. Sustainable Production of African Traditional Beers With Focus on Dolo, a West African Sorghum-Based Alcoholic Beverage. *Front. Sustain. Food Syst.* **2021**, *5*, 2–26. [\[CrossRef\]](#)
51. Cadenas, R.; Caballero, I.; Nimubona, D.; Blanco, C.A. Brewing with starchy adjuncts: Its influence on the sensory and nutritional properties of beer. *Foods* **2021**, *10*, 1726. [\[CrossRef\]](#) [\[PubMed\]](#)

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