EFFECT OF TWO-STAGE FERMENTATION TEMPERATURES AND PARTICLE SIZE ON STABILITY AND SENSORY CHARACTERISTICS OF UMQOMBOTHI

by

THEMBELANI XOLO

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Supervisor: Dr. Z. Keyser
Co-supervisor: Prof. V. Jideani

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DECLARATION

I, **Thembelani Xolo**, declare that the contents of this thesis represent my own work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed **Date**

ABSTRACT

The consumption of Umqombothi, a traditional indigenous pale buff, pinkish-brown to cream-colour after sieving with a yoghurt-like flavour alcoholic beverage produced from sorghum malt and maize malt is widespread in several regions of South Africa. The aim of the research was to determine two stage fermentation and particle size on stability and sensory characteristics of Umqombothi using three different fermentation temperatures namely, U1 (30-30^{o}C), U2 (30-25^{o}C), and U3 (25-30^{o}C), and determine the effect of 24 hours fermentation on the product. Sensory evaluation was conducted on the final products. Three different particle sizes of sorghum malt and maize malt of Umqombothi beer were compared: normal, coarse, and fine powder of sorghum malt and maize malt. Fermented Umqombothi samples were collected before and after the first fermentation, after cooking, at the end of the second fermentation, from the final product, and after fermentation for 24 hours. The microbial content [lactic acid bacteria (LAB), total viable count (TVCs), yeast and moulds (Yeasts), and coliforms] of samples were determined, as well as certain chemical and physical factors [pH, total soluble solids, specific gravity, ethanol, and colour]. The respective final products were subjected to a consumer sensory evaluation based on taste, aroma, appearance, colour, and overall acceptability. Lactic acid bacteria were the most predominant microorganisms throughout the Umqombothi manufacturing process. Sample U1 (30-30^{o}C) had a substantially higher (p < 8.24) LAB count than U2 and U3, which might be attributed to LAB's optimal temperature range, U1 also exhibited significantly (p < 3.45) lowest pH, higher lightness and higher TSS as compared to the U2 and U3. All three fermentation temperatures contained the same percentage of alcohol (2%ABV). Umqombothi brewed at U1 (30-30^{o}C) was also preferred by the consumers. To produce Umqombothi with uniform organoleptic characteristics, it is therefore recommended to perform first and second fermentation at (30-30^{o}C). At this temperature condition, the coarse particle size (sorghum malt and maize malt) produced a significantly (p < 1.44) highest alcohol content and lowest pH levels (3.46). The significantly (p < 4.02) highest overall acceptability was obtained with the normal particle size. Between coarse and normal particle sizes (sorghum and maize malt), there was no significant difference in taste, texture, or colour. The Umqombothi ingredient's fine powder particle size was considerably (p < 0.05) smaller than both the sorghum and maize malts' particle sizes. This difference in particle size had an impact on the development of yeast and LAB during fermentation. Coarse (sorghum malt and maize malt) particle size Umqombothi was the most preferred during consumer sensory evalution. Because of the significantly (p < 4.08) low pH, and significantly (p < 3.44) high alcohol content, coarse particle size was selected to use in subsequent experiments. Laboratory Umqombothi was therefore produced using coarse particle size and 30^{o}C for both fermentation temperature stages, and a consumer sensory evaluation was performed to compare the final product with traditionally preparedUmqombothi.

A smoky flavour was detected by a panelists because Umqombothi prepared in the townships is brewed outside over a wood fire. Laboratory Umqombothi was described as thick and sour with a sweet aftertaste and had the lowest mean overall acceptability of 3.50. With an mean overall acceptability mean of 3.74, Umqombothi collected around Ezimbacwini was described as weak and watery. And Umqombothi collected around Langabuya, had the significantly ($p < 0.05$) highest mean value for appearance, colour, aroma, taste, and texture. Township Langabuya Umqombothi was the most preferred during consumer sensory evaluation.

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DEDICATION

Dedicated to my Heavenly Father, Lord Jesus Christ and to Izinyanya zasekhaya Amanyandube, OJola, and my family; my mother Dideka Xolo, my brother Amos Xolo, for their love, support and believing in me and the Four-Square and last but not the least Ncediswa Cumbe.

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ABBREVIATION/ACRONYMS

Abbreviations/ Acronyms	Definitions/Explanation
ABV	Alcohol by volume
ANOVA	Analysis of variance
˚C	Degree Celsius
CFU	Colony forming units
G	gram (s)
H	hours (s)
KG	Kilo gram (s)
LAB	Lactic acid bacteria
L	Litre (s)
MANOVA	Multivariate analysis of variance
MIN	Minute (s)
ML	Millilitre (s)
MM	Millimetre (s)
RPM	Revolutions per minute
SG	Specific gravity
S	Second (s)
TVC	Total viable count
TSS	Total soluble solids
%	Percentage

GLOSSARY

Term

Back-slopping	This refers to a technique of starting the fermentation by using a small amount of the previous batch into the start of a new batch
Fermentation	The total conversion of sugar to alcohol during beer processing
Natural fermentation	Fermentation during which no starter culture is added. The process is mainly promoted by yeast and lactic acid bacteria
Saccharification	The conversion of soluble starches into sugars using enzymes
Starter	Preparation of living microorganism, which is deliberately used to assist the beginning of fermentation

Syneresis	Is the expulsion or weeping of liquid from a gel, or in this case, gelatinized starch which is often an undesired result in food products
Traditional beer	Umqombothi or other African, ancient, formulated beer prepared from maize, maize malt, sorghum malt, wild yeast, and water
Umqombothi	A beer prepared from maize meal, maize malt, sorghum malt, and water, using tradition method

CHAPTER 1

MOTIVATION AND DESIGN OF THE STUDY

1.1 Introduction

Umqombothi is a B vitamin-rich beverage (Schaepdrijver, 2004:603; Adekoya et al., 2018:23; Hlangwani et al., 2020:7) and is popular with black South Africans, commercially brewed mainly by women and consumed at social gatherings, weddings and rituals (Schaepdrijver, 2004: 589; Dancause et al., 2010: 1124; Hlangwani et al., 2020: 3). The production process involves fermentation or souring, boiling, and fermentation at ambient temperature (Lues et al., 2008:166). The brew's colour varies from pale buff, pinkish-brown to cream-colour after sieving and has a yoghurt-like flavour (Schaepdrijver, 2004:590; Katongole, 2008:13). It has a short shelf life of 2-3 days with an alcohol content of 2-3% (Katongole, 2008:13; Adekoya et al., 2018:21).

The quality of umqombothi may differ depending on the production, utensil and possibly the ingredient; some regions use sorghum malt and maize meal, while others may add brown sugar, brown bread, or other ingredients. , with a pH of between 3.3-3.6, a lactic acid content of 0.26%, total solid of approximately 5-7%, crude protein of 0.7% and mineral salt between 0.18 and 0.36%(Schaepdrijver, 2004:590; Hlangwani et al., 2020:20). Umqombothi is consumed daily in its active state of fermentation, with the composition continuously changing because of the production of carbon dioxide (Schaepdrijver, 2004:590; Dancause et al., 2010:1124; Hlangwani, 2020:5). As the generation of carbon dioxide declines, air gains access to the beer and some alcohol is oxidized into acetic acid, which results in unwanted flavours and aroma. Lactic acid bacteria such as *Lactobacillus* and *Leuconostoc* spp. produce acetic acid during fermentation. Lactic acid bacteria and the yeast *Saccharomyces cerevisiae* are thought to be the predominant microorganisms during fermentation (Simatende et al., 2015:120; Ncube et al., 2020:261; Adekoya et al., 2019:1).

The fermentation temperature is vital to maintain the LAB and *Saccharomyces cerevisiae* in a rapid logarithmic state of growth. The challenge in brewing the ideal Umqombothi is to obtain a balance between a lack of nutrients and the excessive availability of LAB and yeast to provide adequate acid and alcohol, respectively. Excessive nutrients may cause the degradation of starch, resulting in thinning of the Umqombothi. On the other hand, minimal nutrient levels may lead to the production of low levels of acid and alcohol.

1.2 Statement of the research problem

Utilisation of sorghum malt and maize malt throughout South Africa is not extensive. The most common use of sorghum malt and maize malt in South Africa is its consumption as roasted or boiled and futher use in the production of porridge. The production of the traditional alcoholic

beverage "Umqombothi" is limited to traditional occasions because of its short shelf life and constantly changing organoleptic qualities. Traditional beverage is not only a pleasant beverage but also provides valuable nutrients. Umqombothi contains vitamins such as thiamine and riboflavin (B-vitamins) as well as 40% of the daily required niacin need(Lues et al., 2008:164; Konfo et al., 2015:192). As production is generally performed on a household level, the process frequently suffers from inconsistencies with regard to fermentation temperatures and time, inoculum, measuring instruments and precision (Dancause et al., 2010:1124; Konfo et al., 2015:190). Significant effort has already gone into studying the physicochemical characteristics of Umqombothi and identifying microorganisms that are predominantly involved during fermentation (Schaepdrijver, 1998:598; Katongole, 2008:60, Adekoya et al., 2018:2). However, no studies have reported the effect of fermentation temperatures and ingredient particle size on the quality of Umqombothi. Therefore, it is necessary to evaluate the efficency of different fermentation temperatures, and ingredient particle size is necessary to produce Umqombothi.

1.3 Objectives of the research

1.3.1 Broad objective

The study aims were to determine the impact of two-stage fermentation temperature, ingredient particle size, on the microbiology, physicochemical, stability and sensory characteristics of Umqombothi.

1.3.2 Specific objectives

The specific objectives of the research include:

1. Establish the optimal fermentation temperature to produce Umqombothi.
2. Establish the optimal ingredient particle size to produce Umqombothi.
3. Characterize and compare microbial, physicochemical, and stability (syneresis and viscosity) of optimized Umqombothi.
4. Evaluate consumer acceptability of the optimized Umqombothi.

1.4 Hypotheses

It is hypothesized that.

1. Optimisation of processing conditions of temperatures will produce Umqombothi with improve sensory characteristics.
2. It was hypothesized that reducing ingredient particle size will improve the stability of Umqombothi.
3. LAB and *Saccharomyces cerevisiae* are dominant in the relevant microbial community and result in Umqombothi with consistent organoleptic characteristics.

1.5 Delineation

The delineation of the study includes only white star maize meal, plaza maize malt, and king korn malted sorghum. An untrained panel was used for sensory evaluation (consumer acceptance).

1.6 Significance of the research

The study focuses on the factors that influence the quality of Umqombothi, a poor man's drink made by women. The fermentation of beverages is associated with significant benefits for developing countries including increased nutritional value, including protein quality (Simatende et al., 2015:120; Katongole, 2008:2). The brewing process will be simplified, consistent, and convenient as it is optimised. The production of Umqombothi also encourages the use of underutilized grains in the brewing sector and promote their availability to all individuals, resulting in more job opportunities for "woman master brewers". Individuals' lives will be improved, and women empowered. This will increase opportunities for growth and development in rural and low socioeconomic urban areas, as well as contribute to education about the history of traditional brewing in South Africa and the development of new knowledge about this neglected traditional beverage. Because Umqombothi is highly nutritious and ingredients readily available throughout the African continent, the success of this study will also provide food nutrition and sustainability.

1.7 Expected outcomes.

A more stable, consistent, and reproducible product was expected to be the outcome when using a defined fementation temperature and particle size during processing of Umqombothi. Since no information has been documented on optimization (cooking time and fermentation temperature) of the Umqombothi fermentation process and the effect of particle size, new information and processing technology were developed. At least one scientific article will be published in a national or international scientific journal. Furthermore, a Master's of Technology degree at the Food Science and Technology Department of CPUT will be obtained.

1.8 Ethical declarations

The ethical Approval was obtained (205221289/04/2022) from the Faculty of Applied Science of CPUT.

1.9 Thesis Overview

This thesis was compiled in an article format and consists of six chapters. Chapter one introduces the motivation and design of the study, which includes the research problem, objectives, significance of the research, expected outcomes, and contributions of the research. Chapter two is the literature review, which elaborated on the background of the research title,

including various topics such as the background of Umqombothi, the definition of Umqombothi, the importance and development of Umqombothi, traditional uses of Umqombothi, origin and socio-economic impact of Umqombothi, and the composition and benefits of Umqombothi. Chapter three is the first research chapter focused on the physicochemical and microbiological changes during the Umqombothi production process at different fermentation temperatures. Umqombothi was produced using controlled fermentation temperatures to produce three different batches of Umqombothi, following cooking at 60ºC for 40 minutes. The physical attributes (colour characteristics), chemical attributes (pH, ethanol, total soluble solids, specific gravity) and microbiological (enumeration of yeast and moulds, lactic acid bacteria, total viable count and *Enterobacteriaceae*) were evaluated. This chapter further focused on consumer sensory evaluation of the different fermentation temperature scenarios used to produce Umqombothi. Chapter four focused on the physicochemical, microbiological, and chemical attributes of particle size of sorghum malt and maize malt (normal, coarse, and fine powder) of the Umqombothi production process. Umqombothi was tested for physical attributes (syneresis, colour characteristics and viscosity); chemical attributes (pH, ethanol, total soluble solids, specific gravity) and microbiological attributes (enumeration of yeast and moulds, lactic acid bacteria, total viable counts and *Enterobacteriaceae*). This chapter further focused on consumer sensory evaluation of the different particles of ingredients (normal, coarse, and fine powder) to produce Umqombothi. Chapter five is a summary of the entire research project, focusing on the findings, conclusion, and recommendation of the study.

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CHAPTER 2
LITERATURE REVIEW

2.1 Definition of Umqombothi beverage

Umqombothi is an alcoholic drinks are essentially brewed by fermenting sugar-rich extracts, which originate from various plant starches. Today's beverage is brewed from partially germinated (malted) cereal grains which results in the formation of fermentable sugars from starch (Hornsey & Brewery, 2016:345). There are many different types of beverages (clear beer) produced and consumed worldwide, most are different (hops, pure yeast from traditional beverage consumed in Africa (Briggs et al., 2004:589; Hlangwani et al., 2020:2). In developed countries, fermented beverages are produced under controlled scientific conditions, while in Africa traditional customs and age-old techniques are used (Konfo et al., 2015:190; Hlangwani et al., 2020:1; Ncube et al., 2020:256).

Sorghum or opaque beverages are indigenous alcoholic fermented beverages in Africa. Production of these beverages provides social enjoyment, and also has important implications for the food system and economy of the country (Sefa-Dedeh et al., 1999:593; Briggs et al., 2004:598; Kutyauripo et al., 2009:271; Hlangwani et al., 2020:1). These traditional beverages are known by their local names, i.e., Ikagage (Rwanda), Pito or Burukutu (Nigeria and Ghana), Dolo (Burkina Faso), Amgba (Cameroon), Doro or Chibuku (Zimbabwe), Merissa (Sudan), Mtama (Tanzania), Bili Bili (Chad), Umqombothi (South Africa) and Tchakpalo (Ivory Coast, Togo and Benin) (Katongole, 2008:59; Konfo et al., 2015:190; Coulibaly et al., 2020:194; Hlangwani et al., 2020:1). Malted sorghum, millet, or maize are used to prepare traditional African beverages, and they are much thicker than clear beer with a distinctive sour yoghurt-like refreshing taste (Adekoya et al., 2018:21; Bayoï & Etoa, 2021:75). Many solid particles cause these beverages to be opaque and pink-brown. The alcohol content of indigenous beverages varies typically between 2 and 4% (Hlangwani et al., 2020:6). In 1951, in mining areas in South Africa, traditional beverage used to be referred to as Umqombothi,exhibiting a high nutritional content (Lues et al., 2008:164).

Due to the brewing method used (spontaneous fermentation and poor hygiene), Umqombothi has a short shelf life, which limits its consumption to a day or two after production, and hence its availability is limited (Katongole, 2008:13; Cason et al., 2020:2).

In the past, concerns have been raised to South African authorities with regard to the microbiological content of Umqombothi, the possible presence of toxic compounds, and general hygienic practices during brewing (Lues et al., 2008:165; Hlangwani et al., 2020:2). Lactic acid bacteria and yeast are the predominant microorganisms involved during fermentation (Simatende et al., 2015:120; Mogmenga et al., 2019:647), with LAB primarily responsible for souring the product and yeast for alcoholic fermentation (Hesse, 2015: 190; Konfo et al., 2015:190; Coulibaly et al., 2020:612). Traditional African beverages are different from European beers; malt milling is the early step in the brewing process to promote enzyme

activity, unlike traditional beers (Mousia et al., 2004:2214). The early step in the brewing operation, which has not received the attention it deserves in Umqombothi production process is milling, milling grains enhances the enzymatic hydrolysis of starch. Polymeric molecules such as starch, proteins, and starch cell walls are converted into low molecular weight compounds by these sorghum malt and maize malt enzymes.and have a marked effect on fermentation of wort sugars, hence the end product quality (Mousia et al., 2004:2213; Konfo et al., 2015:191). Significant effort has gone into determining the physicochemical characteristics of Umqombothi and identifying the microorganisms that are involved during fermentation (Schaepdrijver, 1998: 592; Katongole, 2008:60, Adekoya et al., 2018:23). Umqombothi a traditional indigenous pale buff, pinkish-brown to cream-colour after sieving (Figure 2.1).

Figure 2.1: The final product of Umqombothi, a popular traditional beer of South Africa

2.2 The origin and social-economic role of Umqombothi

Umqombothi is a traditional beverage made from a combination of maize meal, crushed corn malt, sorghum malt, water, and yeast (Schaepdrijver, 2004:593; Van Wyk, 2011:858; Adekoya et al., 2018:22), originally prepared from the fleshy root of *Glia gummifera* (moerwortel plant). Historically, several root species have been employed in brewing. This method became completely obsolete, and the roots were substituted with industrial yeast or backslope. Umqombothi is brewed following traditional customs, and these vary slightly between regions (Briggs et al., 2004:589; Ncube et al., 2020:256). The preparation of Umqombothi beverage is

a tradition preserved by African women brewers and is passed down to the next generation (Coulibaly et al., 2020:195; Hlangwani et al., 2020:2). Hand-mixing sorghum malt, maize malt, maize meal, yeast and water takes place in an a iron-cast pot or plastic bucke/drum. The elderly women, use previous experience and basically “gauge” the weight by hands or bowl with mixed ingredients are soaked overnight for spontaneous wild yeast fermentation. The beverage is traditionally prepared over a fire outside the house. The brew is stirred using an iphini, a traditional wooden spoon. Intshwela is the sediment at the bottom of the cooking pot that is added to the strained beverage to enhance flavour. The product can also be consumed as porridge (isidudu) (Katongole, 2008:60; Dancause et al., 2010:1124). Following this is chilling in a stainless steel or plastic bowl outside the home, followed by secondary fermentation, predominately perfomed by LAB in a plastic bucket or cast iron pot covered by blanket. In order to provide a warm fermentation environment during the winter, the fermentation containers are set on top of the cow waist product known as "ubulongwe" and covered with blankets or a fire (Hlangwani et al., 2020:4). The progress of the fermentation process was tested by lighting a match close to the pot, and if the match blows out quickly, the brew is ready. Following the fermentation, the mash is filtered through a tube-shaped, woven, grass strainer (intluzo) (Briggs et al., 2004:593; Hlangwani et al., 2020:5). Finally, the grain that remains in the sieve is squeezed out and placed on the ground for chickens. While casting the corn, the brewer typically expresses gratitude to their forefathers. Sewing multiple strands of properly prepared, twisted, grass-like sedge stems together creates the strainers. The strainer “Intluzo” was an essential item in a traditional Xhosa household, and it was given as a wedding gift to the newly married woman. The beverage is then sieved and placed into a huge plastic drum or iron cast pot, and served with Ukhamba for sharing with friends and relatives..

The production and marketing of Umqombothi bevarge remain the women's responsibilities, from which they derive a substantial income. Umqombothi is sold and consumed in urban and rural areas. This beverage is also used in religious ceremonies such as marriage, praying for rain, communication with ancestors, births, the handing over of a dowry, initiation school, and burial ceremonies (Lues et al., 2008:164; Katongole, 2008:60; Dancause et al., 2010:1124; Hlangwani et al., 2020:20). After community work or meetings of mutual associations, Umqombothi is consumed to provide energy. Figure 2.2 illustrate the traditional production process of Umqombothi.

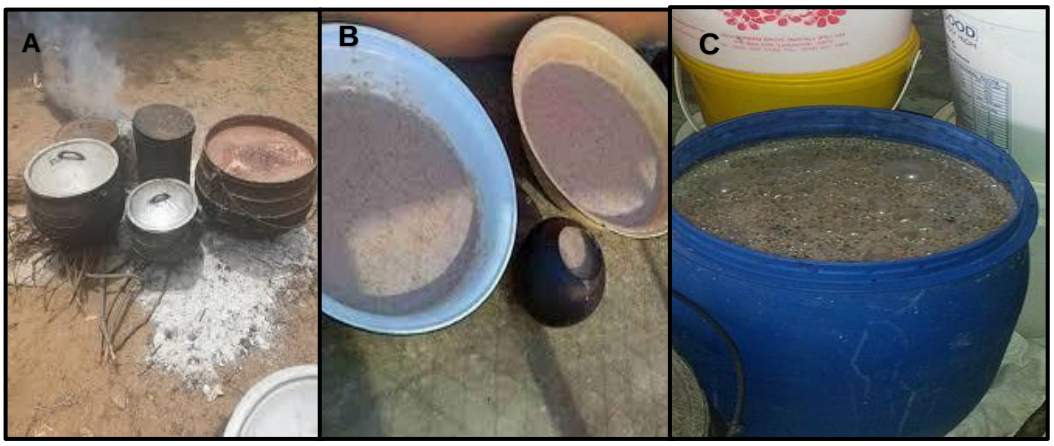

Figure 2.2: The traditional indigenous production process of Umqombothi: A, Cooking; B, Cooling and C, fermentation.

2.3 Different types of fermentation

Fermentation refers to biological processes resulting in desirable modification of ingredients through enzymatic rather than microbial action. Impact characteristics such as aroma, flavour, texture, and nutritional profile in beverages result from fermentation (Mensah, 1997:272; Terefe & Food, 2016:1). Indigenous traditional beverages are produced through fermentation in different parts of Africa, using various manufacturing techniques and raw materials (Usai et al., 2013:146). Traditional African beverages are mostly dominated by spontaneous fermentation of alcoholic fermentation and lactic acid fermentation. There are four different fermentation processes. 1) Yeast is the main organism involved in ethanol generation during alcohol fermentation. 2) Lactic acid bacteria produce substances such as lactic acid, acetic acid, ethanol, carbon dioxide, and hydrogen peroxide that prevent infections and spoilage germs from growing. 3) Acetic acid is produced by *Acetobacter* spp., through the oxidation of alcohol. 4) Alkaline fermentation often occurs during the fermentation of products with high protein content such as fish, soybeans, and locust beans and the involvement of *Bacillus* spp. (Mensah, 1997:272; Katongole, 2008:6; Terefe & Food, 2016;1). The proteins of the raw materials are broken down into amino acids and peptides and ammonia is released, which raises the pH value.

Therefore, the fermented products varying in quality depending on the type of raw materials, environmental conditions, and containers used (Briggs et al., 2004:589; Simatende et al., 2015:120; Adekoya et al., 2018:21). During the production of these indigenous beverage, starch is required as a source of sugar and as a thickening and suspending agent. The gelatinization of starch, which gives beverage its characteristic creamy body, also helps to keep the grain and malt particles, which are crucial components of the beverage, suspended (Schaepdrijver, 2004:85; Konfo et al., 2015:191). A small particle (fine powder) of cereal grain,

a starch granule, and suspended yeast causes African beverages to be opaque. Gelatinization and incomplete degradation of starch are what cause African beverages to be high in viscosity (Brookes, 2004:86; Lyumugabe et al., 2010:209; Konfo et al., 2015:191).

The art of brewing is to provide enough nutrients for LAB and yeast to grow but also to avoid degradation of the starch, which will result in a thin beverage. Since the traditional fermentation process is performed under uncontrolled temperature conditions and unhygienic environments it is a challenge to consistently produce beverage with long shelf life (Lyumugabe et al., 2012:510; Konfo et al., 2015:192; Adekoya et al., 2019:1). Umqombothi is a typical example of lactic acid fermentation followed by alcoholic fermentation in which initially, LAB and later yeasts play the dominant role (Usai et al., 2013:146; Simatende et al., 2015:120). The brewing process of traditional African beverages has received significant advancement in its scientific knowledge and technology over the last century. In the current malting and brewing sector, there is a vast spectrum of technological, biochemical, biological, and genetic advancements (Nyamunda et al., 2018:390). Significant effort has already gone into the physicochemical characteristics of traditional African beverages and identifying microorganisms that play a part in fermentation (Briggs et al., 2004:593; Katongole, 2008:60). Umqombothi is known to provide the needed vitamins of thiamine and riboflavin (B vitamins) and 40% of the recommended daily requirement of niacin (Lues et al., 2008:164; Adekoya et al., 2018:22).

2.4 Ingredients of Umqombothi

2.4.1 Maize

One of the most important grain crops in South Africa is maize (*Zea mays*), which is the main staple food for approximately 50% of the population. It is used for human consumption in the form of porridge, breakfast cereals, and snacks (Pretorius & Schönfeldt, 2013:520). White maize meal is fortified with vitamin A, iron, zinc, folic acid, thiamine, niacin, vitamin B6, and riboflavin as part of an initiative of the Department of Health in South Africa (Pretorius & Schönfeldt, 2013:520). The endosperm of maize grain consists of about 71% starch, and the endosperm consists of zein protein that is soluble in alcohol (Sofi et al., 2009:244). Maize starch has a higher gelatinization temperature than barley and wheat, and it cannot be converted into simple sugars between 63–67^{o}C, temperatures must exceed 100^{o}C to ensure endosperm disruption (Yeo & Liu, 2014:1613). Maize malt and fortified commercial maize meal are the primary ingredients in the production of South African traditional Umqombothi.

2.4.2 Sorghum malt

Sorghum belongs to the grass family *Gramineae,* and it contains minerals, vitamins, protein, and micronutrients that are important for human health. Sorghum has a high starch content, and the crop is resistant to extreme environmental stress (Yeo & Liu, 2014:1612; Motlhaodi et

al., 2018:123). Hydrobenzoic acid, hydroxycinnamic acid and flavonoids are polyphenolic compounds that have been identified in sorghum grains. Antioxidant activity and phenolic compounds are present in the pericarp layer of sorghum grain. These activities are correlated to beneficial health properties such as antimicrobial, reduction of oxidative stress, and anti-cancer activity (Rao et al., 2018:1; Rumler et al., 2021:1).

2.4.3 Water

The quality of all ingredients used during brewing affects the taste of the final product, therefore, potable water must be used (Papazian, 2006: 42; Olajire, 2020:2). The production of traditional beer involves the mixing of the raw ingredients with water. The water content of beer is 90-95% (w/w) (Bamforth, 2006:443; Papazian, 2006:42; Olajire, 2020:2). In low socio-economic communities' water is mainly obtained from dams, rivers, and commercial rainwater tanks.

2.5 Milling of grains and the effect of particle size

One of the purposes of milling is the breaking of cereal grains to an appropriate particle size, which will facilitate water penetration during subsequent cooking processes (Russel, 2003:9). The optimum size of the ground particle is a subject of disagreement. Some engineers think the particle should be as tiny as possible to provide the water the most access for the starch to be hydrolyzed, while others think larger particles would offer a higher yield since the cooking can work on the larger particles. . The key is not to ground the starch so fine to cause problems in co-product recovery, but to expose the starch (Russel, 2003:11). A wide variety of milling equipment is available, and the milling technique depends on the method of mashing and separation that will be used (Russel, 2003:9; Willaert, 2006:446).

Roller mills are used during the preparation of South African traditional beverage such as Umqombothi. In a roller mill, the cereal is nipped as it passes through the rollers. The rollers operate at different speeds to apply a shearing force in some instances. For the starch endosperm to be better accessible to malt enzymes and improve the extraction process, milling of the malt must be performed (Russel, 2003:10; Willaert, 2006: 446; Niemi et al., 2012:155) The final particle size produced by this method depends on the material being processed (Drakos et al., 2017:326). The husk is more flexible and will be less affected during roller milling, and it may be utilized to build up the filter bed (Willaert, 2006:446). Enzymatic conversion of starch, proteins and starch cell walls to low molecular weight compounds influences the fermentation of wort sugars and hence the quality of the final product. The hydrolysis of starch is catalysed by enzymes and results in the formation of oligosaccharides and small molecules such as sugars (Russel, 2003:13; Mousia et al., 2004:2213). The cooking and saccharification process hydrolyse starch into fermentable low molecular weight sugars, such as glucose, sucrose, fructose, maltose, and dextrin (Russel, 2003:15; Willaert, 2006:447).

Amylase releases glucose, which is then followed by the exoenzyme glucoamylase (amyl alpha glucosidase). Yet, in order for the amylase to have access to the starch, the granular structure of the starch must first be broken down by gelatinization. (Drakos et al., 2017:326).

When the meal-water slurry is heated, the starch granules begin to absorb water and expand. They gradually become large and lose their crystalline structure. A mash's highest viscosity is likewise its peak of gelatinization. (Russel, 2003:11). According to Moussa et al. (2004:2214), starch amylases do not occur evenly for small and large wheat granules. At a higher temperature, large starch granules gelatinize earlier, despite smaller granules having a slightly lower gelatinization temperature. However, starch hydrolysis strongly depends on the physical state of starch since amorphous starch regions are more accessible for enzymes to attach and hydrolyse the starch than crystalline regions. Therefore, starch gelatinization plays a vital role in the conversion of starch since it improves the susceptibility of starch to enzyme hydrolysis. Saccharification is the complete degradation of starch to maltose and dextrin (Willaert, 2006:448; Drakos et al., 2017:326). Starch α-amylolysis also depends on the particle size of starch granules. According to Mousia et al. (2004:2214) at a low temperature of approximately 35^{o}C, small starch granules are hydrolysed faster than large ones. However, at a higher temperature of 65^{o}C large granules were more susceptible than small ones. This is due to the early gelatinization of the large granule.

2.6 Composition and benefits of Umqombothi

Umqombothi is mainly produced under uncontrolled conditions in most parts of South Africa (Ncube et al., 2020:256). Formally, the sensory, physical, physicochemical, chemical, and microbiological evaluations are the fundamental subcategories of the qualitative aspect of brewing. The main qualities of beer characteristics are mouthfeel, aroma, appearance, and flavour. The primary responsibility for these qualities is the type of raw material used and the fermentation process (Briggs et al., 2004:590; Katongole, 2008:13; Hlangwani et al., 2020:3).

2.6.1 Physicochemical properties of Umqombothi

The colour of the Umqombothi brew is determined by the presence of suspended solid particles. The colour varies from pale buff, pink-brown to cream after sieving, and the beer has a yoghurt-like flavour (Briggs et al., 2004:590; Katongole, 2008:59; Adekoya et al., 2018:22). The high calorie of the beer is determined by the suspended solids (5–7%), typically starch residue, dextrin and dietary fibre (Hlangwani et al., 2020:6). The beer has a short shelf life of 2-3 days with an alcohol content of 2-3% (Katongole, 2008:13; Adekoya et al., 2018:22; Hlangwani et al., 2020:7). The physicochemical properties of Umqombothi differ from region to region, with a pH of 3.3-4.2, a lactic acid content 0.02–0.26%, total solid approximately 6%, crude protein of 0.7% and mineral salt 0.18-0.36% (Briggs et al., 2004:590; Coulibaly et al., 2020:195; Hlangwani et al., 2020:6). Umqombothi has a creamy distinctive sour taste and fruity

odour. The sour taste is caused by LAB and organic acid formation during fermentation. The fruity flavour is caused by diacetyl formation from the wild-type strain *Saccharomyces* species (Hlangwani et al., 2020:7).

2.6.2 Nutritional benefits of Umqombothi

Umqombothi is an excellent energy source for most African people, a calorie-rich beverage with 130-394 kJ/100 g, and is especially consumed in low socio-economic communities. Umqombothi is also rich in B-group vitamins such as thiamine (B_1), riboflavin (B_2), niacin (B_3), folic acid (B_9), and vitamin C, which is contributed by the raw materials and the fermentation process (Briggs et al., 2004:598; Hlangwani et al., 2020:7). These vitamins are essential for genomic and non-genomic methylation and clearance of homocysteine. Umqombothi also contains essential amino acids (EAAs) such as leucine, lysine, phenylalanine, and tryptophan, as well as non-essential amino acids (NEAAs) such as aspartic acid, and glutamic acid (Tokpohozin et al., 2019:182; Hlangwani et al., 2020:7). Leucine, an amino acid required for ATP production and protein synthesis, is abundant in Umqombothi. Amino acids assist in building lean muscle, boosts mitochondrial biogenesis, improve fertility and metabolism, and avoid sleeplessness. Umqombothi also contains probiotics, thereby boosting gut health, bowel movement, and antimicrobial mechanisms (Hlangwani et al., 2020:7).

2.7 Sensory description of Umqombothi

2.7.1 Overall impression

Umqombothi is a viscous beverage and it has an inherent grittiness obtained from the maize used. It is an opaque beverage with a creamy brown colour due to the sorghum that is used. The beverage also has naturally high carbonation, which is mainly influenced by the fermentation rate. A slightly sweet taste characterizes sorghum beverage due to the fruity flavour, a sour taste due to lactic acid, and a mild bitterness on the tongue (Yeo & S. Q. Liu, 2014:1613; Adekoya et al., 2018:22).

2.7.2 Aroma

Umqombothi has a strong sorghum malt aroma (grassy, husky, dust-like) with a hint of lactic acid sourness due to the LAB. They also give off the aroma of cooked maize but slightly due to the yeast providing some fruity/yeasty notes. When the beverage is freshly produced, it gives off substantial carbon dioxide aromas of fermentation, indicating the active state of the fermentation process that the beverage is usually consumed in, and once the process ends or fermentation stops, it gives off rotten meat or sulphur aromas indicating yeast autolysis or that the beverage is old or spoiled (Briggs et al., 2004:593).

2.7.3 Appearance

The beverage appearance may differ depending on the sorghum and maize varieties used for production. The final colour of the product may range from a light brown, creamy-white to creamy pink-brown. The foam colour will be the same as the beverage, and the foam head will differ with the age of the beverage, where the fresh beverage will have extensive and lasting foam while the old beverage will have a small to almost no foam head that usually dissipates fast. Also, some dark or precipitate may be visible from the sorghum malt hull that remained in the beer post-straining (Briggs et al., 2004:593; Katongole, 2008:59).

2.7.4 Flavour

For first-time tasters, the sourness of Umqombothi may be overpowering. For this reason, it is encouraged to have two or more initial small mouthfuls of beverage. Thereafter, a big mouthful of beverage should be taken and swirled around in the mouth before swallowing. A slightly cooked maize porridge and a strong sorghum malt flavour should be present, but this should not be burnt or overcooked. The malt flavour can be described as earthy, grassy, husky, and dusty. The beverage should also have a clean sour (lactic acid) taste some will describe the sour as a sour bite, but it should never be overpowering. The beverage may also taste sweet, when it tastes overpowering with an acetic acid flavour, it indicates that the beverage is old, with flavours originating from the fermentation that slows down (Briggs et al., 2004:590). A strong acid taste can also originate from contamination with acetic acid bacteria with consequent oxidation of sugars and alcohol and the formation of acetic acid. Ultimately, the grassy, malty, and sour flavours should be in balance. The mouthfeel ranges from a viscous feel such as Amahewu or drinkable yoghurt to almost a non-viscous (watery) beverage. Due to the maize porridge ingredient, it is a mouth-filling beverage, but not difficult to swallow. The maize porridge contributes to the grittiness of the beer and can be reduced by straining the beverage before consumption (Hlangwani et al., 2020:3).

2.8 Microbiology of Umqombothi

The microbiology of many African traditional beers is quite complex, and fermentation is natural and involves mixed cultures of yeast, bacteria, and fungi (Katongole, 2008: 8; Hlangwani et al., 2020:9). *Saccharomyces cerevisiae* is the yeast species that dominate in producing alcoholic beverages worldwide. Strains of this species contribute to alcoholic fermentation, flavour, and aroma and maintain beverage sensory qualities (Yeo & Liu, 2014:1610; Walker & Stewart, 2016:3; Adekoya *et al.*, 2019:1). These strains of *S. cerevisiae* traditionally conduct top fermentations where yeasts congregate on the surface of the fermenting wort (Lodolo et al., 2008:1022). Umqombothi is a typical example of lactic acid fermentation followed by alcoholic fermentation in which initially LAB and later yeasts play the dominant roles (Usai et al., 2013:146; Simatende et al., 2015:120; Adekoya et al., 2018:21). Bacteria typically

dominate the early stages of fermentation due to their higher growth rate (Hlangwani et al., 2020:9).

Fermentation with LAB is not an exact science. One brand of beer may have different levels of sourness from batch to batch (Spitaels et al., 2015:24; Pejin et al., 2018:154). Bacteria play several beneficial roles in the production of beers (e.g. in the preparation of acidulated malt), while some beer styles (e.g. Berliner Weissbier, Lambic beers and sorghum beers) depend on the action of bacteria during the production process (Menz et al., 2010:14; Vriesekoop et al., 2012:339). Diacetyl is produced by some LAB and contributes to the flavour and aroma profile of beer and is associated with a buttery or butterscotch flavour. In some beers, this flavour is unacceptable, but for others, it is desired (Pejin et al., 2018:153).

Fungal genera present in beer are mainly representatives of *Aspergillus, Penicillium,* and *Trichothecium.* Fungal species have been found in Umqombothi produce in Eastern cape and Gauteng. Fungal are considered as before and after harvest contamination (Katongole, 2008:8; Adaku et al., 2019:23). Off flavours are produced by microbes present in the utensils and not from the raw material. *Acetobacter* species promote the formation of volatile acid, and it reduces the shelf life of Umqombothi (Hlangwani et al., 2020:2).

2.8.1 Saccharomyces cerevisiae in sorghum beverage

The most economical biotechnologies used for the fermentation of carbon sources into alcohol by yeast are among the most important and oldest. Brewing yeast is a member of the *Saccharomyces* genus. Yeast strain selection is essential to maximize alcohol and beverage sensory qualities (Venturini Copetti, 2019:58; Walker & Stewart, 2016:3).. Bottom fermentation is conducted by a strain of *Saccharomyces pastorius* and tends to flocculate (Gallone et al., 2018:148; Walker & Stewart, 2016:3; Venturini Copetti, 2019:58).

The yeast species that dominate in the production of traditional indigenous beverage, e.g., Umqombothi, is *Saccharomyces cerevisiae*. Particular strains of this species influence the flavour and aroma characteristic of Umqombothi (Briggs et al., 2004:149; Katongole, 2008:8; Adekoya et al., 2018:25). In most African traditional indigenous alcoholic beverages, a pure strain of *Saccharomyces cerevisiae* is used as the inoculum during fermentation to increase the shelf life of the products. To address the challenges of sensory properties quality variability and microbiological safety in traditional African fermented drinks, the use of starter cultures was proposed as a potential strategy for fermentation control and optimization. The inclusion of antagonistic or competitive microorganisms or their metabolic products together with *Saccharomyces cerevisiae* in starter cultures can prevent or inhibit the growth of undesirable microorganisms during fermentation. These strains are either isolated from the product or obtained from yeast production companies (Sefa-Dedeh et al., 1999:594; Kutyauripo et al., 2009:272; Lyumugabe et al., 2012:510; Konfo et al., 2015:192).

2.8.2 Benefits and application of Saccharomyces cerevisiae as a starter culture in Umqombothi

Indigenous fermented beverages play a significant part in African heritage. Most of these beverages are still produced by spontaneous fermentation at household and semi-industrial scales, e.g., Umqombothi. The Umqombothi fermentation process is not described in detail in the literature due to uncertainties in the process with regards to equipment and uncontrolled hygiene and fermentation conditions (Katongole, 2008:30; Hlangwani et al., 2020:2). To maintain the production of Umqombothi, sustainable changes to the production process must be implemented, including a change from spontaneous to controlled fermentation, upscaling of the production and the introduction of a purified starter culture. According to Adekoya (2019: 2), *S. cerevisiae* is one of the most dominant organisms in the spontaneous fermentation of Umqombothi and the most critical microorganism in quality and economics (Russel, 2003:86). It is the only living microorganism that can switch from respiratory to fermentation mode. If glucose is present, it will always take a fermentative route to utilize it (Russel, 2003:86). Yeast can effectively absorb sugars from the surrounding fermentation medium. The yeast cell size varies with the cycle stage and age of the cell and is approximately 5-10 µm in length (Russel, 2003: 86; Walker & Stewart, 2016:4; Gallone et al., 2018:148). Katongole (2008:30) mentioned that *S. cerevisiae* isolated from African-fermented beer products are different from the well-recognized starter culture. He demonstrated that during the preparation of indigenous fermented beverages, a starter culture isolated and purified from the original product should be used. The yeast strain must be selected according to the technical properties required for the actual type of production. Factors affecting the efficiency of the yeast strain for the specific application will include the optimal temperature, the ability of the yeast to survive high ethanol concentrations, and the flavour compound of interest or concern. A careful choice of a particular strain will lead to successful fermentation. Table 2.1 illustrate some of the yeast function in Africa indigenous traditional beverage.

2.8.3 Lactic acid bacteria in sorghum beverage

Sorghum beer is the product of alcoholic fermentation by top yeast fermentation, mostly *Saccharomyces cerevisiae* and LAB. The lactic acid fermentation in sorghum beer is affected by microorganisms inherent in raw material, containers, and the surrounding environment (Briggs et al., 2004:593; Adekoya et al., 2018:23). Spontaneous fermentations are challenging to control and are not predictable in terms of the length of fermentation and quality of the product.

Table 2.1: Functions of yeast in African indigenous fermented beverage products (Katongole, 2008:51)

Product Type	Yeast Function	Compound
fermented foods and beverages	Fermentation of carbohydrates	Alcohols production
	Production of aroma compounds	Esters, alcohols, organic acids, carbonyls production
	Stimulation of lactic acid bacteria	Essential yeast metabolites production
	Inhibition of mycotoxin-producing moulds by nutrient competition, production of toxic compounds	Prevention of aflatoxin production
	Degradation of mycotoxins	Prevention of mycotoxin production
	Linamarase activity	Degradation of cyanogenic glycosides
	Production of tissue degrading enzymes	Cellulases and pectinases
	Probiotic properties	Lactic acid producing bacteria

They can produce an unwanted product, a product with a short shelf life, and sometimes not safe since they are prone to contamination by pathogens (Briggs et al., 2004:590; Lues et al., 2008:165). The predominant LAB in South African sorghum beer, Umqombothi, are *Leuconostoc*, *Lactobacillus, Streptococcus, Pediococcus, Micrococcus,* and *Bacillus* species (Katongole, 2008**:**8). Various brands of sorghum beer are produced in different cities in South Africa, namely Isiqatha, Utshwala, Imfulamfula, and Umqombothi. Umqombothi is considered a nutritious product because it contains a mixture of organic acid, alcohol, vitamins, and other growth factors produced by LAB (Schaepdrijver, 2004:593; Hlangwani et al., 2020:1). Lactic acid bacteria are involved in the most biological changes that occur during brewing. Souring occurs first because of LAB, while alcohol and lactic acid fermentation occur last. The first acidification is due to inherent LAB in raw material and equipment, and the second is due to the addition of fermented sorghum yeast or back slope (Kutyauripo et al., 2009:272).

2.8.4 Benefits and application of LAB as a starter culture in Umqombothi.

Lactic acid bacteria fermentation has been used to naturally improve and fortify substrates, e.g., milk, vegetables, and cereal beverages. The United States Food and Drug Administration has awarded Generally Recognized as Safe (GRAS) certification to several of these goods (amasi, Lambic beer, cheese), (US FDA) (Peyer et al., 2016:18:18). Genera of *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococus*, and *Streptococcus* are naturally present in the pericarp of most grains, equipment, utensil, and humans. Therefore these microorganisms are suitable to use in the preparation of fermented cereal beverages such as sorghum beer (Peyer et al., 2016: 18). Fermentation with LAB has improved protein digestibility and enhanced the products' organoleptic qualities. According to Peyer (2016:18), the *Lactobacillus* spp. adapt

faster than other species to the conditions in malted cereals and enter the exponential growth phase sooner. Sensorial and textural properties of liquid cereal substrate improve during fermentation with LAB (Peyer et al., 2016:19). In the attempt to guarantee product safety and functionality of sorghum beer without changing the sensorial characteristics, research has aimed to replace the natural back slope culture with a defined starter culture containing single or mixed LAB strains. The use of a defined starter culture also produced beer with a better appearance, aroma, texture, and acceptability than the original traditional beer (Sefa-Dedeh et al., 1999:594; Konfo et al., 2015:194; Adinsi et al., 2017:19).

A defined LAB and *S. cerevisiae* starter culture could stabilize the organoleptic quality of Umqombothi and increase its ethanol content. Starter cultures have been applied successfully to many indigenous traditional beers in Africa. *Pito* beer contains a higher ethanol content than traditional *Pito* produced in the laboratory. Satisfactory production of a *Pito* beer with a taste and aroma similar to local *pito* beer was produced from *S. cerevisiae* combined with *Lactobacillus plantarum* as a starter culture (Lyumugabe et al., 2012:526). *Sacccharomyces cerevisiae* in combination with *Candida. tropicalis* as a starter culture was successfully applied in the production of the alcoholic fermented beer *Tchapalo. Dolo* beer, however, had a taste and aroma that did not differ significantly from the local *dolo* beer, and was produced from starter culture combinations of one strain of *L. fermentum* and two *S. cerevisiae* strains (Sefa-Dedeh et al., 1999:595; Lyumugabe et al., 2012:526).

Microorganisms involved in the spontaneous fermentation of sorghum beer are very diverse (Leuconostoc, Lactobacillus, Streptococcus, Pediococcus Aspergillus, Paecilomyces, Cladosporium, Fusarium and Penicillium). Therefore, research needs to be broadened to other fermentation methods applicable to sorghum beer. Factors to consider include (i) the souring and alcoholic fermentation steps during traditional home brewing in South Africa are not separated (Katongole, 2008**:**8; Usai et al., 2013:146), (ii) an incubation temperature that would encourage the pure culture of LAB and suppress other organisms during the first phase of fermentation needs to be maintained (Briggs et al., 2004:598), and (iii) chilling of the wort at 14-16^{o}C and pitching with a pure yeast strain could slow the fermentation and lengthen the shelf life of the beer (Schaepdrijver, 2004:593). Table 2.2 illustrate some of the lactic acid bacteria involve during the fermentation of cereal product.

2.8.5 The role of fungal toxins (mycotoxins) in fermented beverages

In Africa, adults and infants on a daily basis consume large amounts of fermented beverages and porridges prepared from cereals such as maize, sorghum, and locust beans (Adekoya et al., 2019:1). Maize, cassava, wheat, and barley are part of an extensive range of agricultural commodities that can be contaminated by mycotoxins (Lulamba et al., 2019:184; Nout, 2014:175; Venturini Copetti, 2019:58).

Table 2.2: Lactic acid bacteria genera involved during fermentation of cereals (Katongole, 2008:54)

Genera of LAB	Cell form	Catalase activity (+/-)	Gram-stain (+/-)
Lactobacillus	Rods (Bacilli; coccobacilli)	-	+
Streptococcus	Spheres in chains (Cocci)	-	+
Pediococcus	Spheres in tetrads (Cocci)	-	+
Lactococcus	Cocci	-	+
Leuconostoc	Spheres in chains (Cocci)	-	+
Bifidobacterium	Branched rods	-	+
Carnobacterium	Cocci	-	+
Enterococcus	Cocci	-	+
Sporolactobacillus	Rods	-	+
Lactosphaera	Cocci	-	+
Oenococcus	Cocci	-	+
Vagococcus	Cocci	-	+
Aerococcus	Cocci	-	+
Weisella	Cocci	-	+

When swallowed, mycotoxins are poisonous and produce carcinogenic secondary metabolites of mycotoxigenic fungus (Burger et al., 2013:57). Mycotoxigenic fungi can infiltrate deep into cereal crop matrices and produce mycotoxins during the pre-harvest, storage, transportation, processing, and marketing stages. Toxicologically significant mycotoxins include aflatoxin B_1 (AFB_1) create by *Aspergillus* spp., and fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), fumonisin B_3 (FB_3), deoxynivalenol (DON) and zearalenone (ZEA) produced by *Fusarium* spp. These toxins cause a variety of biochemical effects, including carcinogenic, mutagenic, teratogenic, estrogenic, neurotoxic, hepatotoxic, nephrotoxic, cytotoxic, and immunosuppressive conditions. Aflotoxin B_1 is mainly produced by *A. flavus* and *A. parasiticus* and poses a serious threat to human and animal health by causing hepatotoxicity, teratogenicity, immunotoxicity as well as liver cancer, and is classified a Group 1 carcinogen by the International Agency for Research on Cancer (IARC). The fumonisins are mainly produced by *F. verticillioides* and *F. proliferatum,*causes a decrease in complex sphingolipids, glycerophospholipids, and

cholesterol, which are essential for cell membrane integrity, resulting in possible intestinal epithelial cell proliferation, disruption of cytokine production and modulation of intestinal barrier function. Fumonisin B has been associated with neural tube defects, stunting in children and oesophageal cancer, and is classified a Group 2B carcinogen. Deoxynivalenol, a vomitoxin, is produced by fungi belonging to the *F. graminearum* spp. complex. It causes intestinal barrier impairment and immunostimulatory effects in low doses in animals and emesis, reduction in feed conversion rate, and immunosuppression in high doses. Contamination by ZEA is mainly caused by *F. graminearum, F. equiseti, F. culmorum, F. cerealis* and *F. semitectum.* ZEA is an estrogenic mycotoxin affecting male and female reproductive systems. Chronic exposure to mycotoxins, such as DON and FB in combination, has been suggested to modulate child growth by inducing a poor appetite, gut impairment, inflammatory diarrhea, decreased nutrient absorption and systemic immune activation. Co-exposure to multiple mycotoxins can have additive effects, contributing to existing health conditions and disease burden.

Mycotoxigenic *A. flavus, A. parasiticus*, and *F. verticilliodes* have been identified in several studies on Umqombothi in South Africa (Adekoya et al., 2018:22; Mutenje et al., 2019:126). Mycotoxins levels have also been determined in traditional beer consumed in KwaZulu Natal (n=11), Transkei (n=18) and Gauteng (72 µg/L). The most common pathogen was Aspergillus flavus (26%. (Adekoya et al., 2018:23). Mycotoxin contamination cannot be ruled out in opaque beverage since the production environment often supports fungal colonization. Studies have reported fermentation as the process that reduces mycotoxin levels in cereals. Methods for prevention of chronic exposure, particularly in low socio-economic communities, remain critically important (Cardello et al., 2016:23). In developed countries high standards of the major food suppliers and retailers are upheld and the regulatory controls deter the importation and marketing of contaminated products. In developing countries, a limited number of countries have legislative maximum levels for mycotoxins in food and beverages, and implementation thereof is often poor. Effective reduction of mycotoxins in cereal grains has been demonstrated with hand sorting, flotation, washing, dehulling of maize kernels and combinations thereof *in vitro* and field studies. Dehulling and shelling of maize are common practices in West Africa, with the removal of the pericarp an effective way to reduce mycotoxin contamination. In various African nations, including Benin, Nigeria, Tanzania, South Africa, and Malawi, the efficiency of hand-sorting maize by eliminating obviously sick and broken kernels resulted in a large decrease of fumonisins.

2.9 Safety challenges with Umqombothi production process

Umqombothi remains one of the oldest and most famous traditional beverages amongst black South Africans. The grains used are locally available and grown by local farmers, since the fermentation of Umqombothi is done under uncontrolled temperature conditions and relies on natural (wild) fermentation with poor hygienic conditions, making a safe and consistent

beverage with a longer shelf life is almost impossible (Lyumugabe et al., 2012:510; Konfo et al., 2015:191; Adekoya et al., 2019:1). Several studies reported the presence of mycotoxins. In Umqombothi, the most common diseases associated with mycotoxicosis are nephropathy, various types of cancer, alimentary toxic aleukia, hepatic diseases, various haemorrhagic syndromes, immune and neurological disorders, and a significant reduction in mycotoxin levels that occurred during fermentation. The levels of eight mycotoxins [(B1 (FB1), B2 (FB2), B3 (FB3), aflatoxins (AF), fumonisins (FB), and deoxynivalenol (DON)] were determined in traditional maize-based beverage consumed in Kwazulu-Natal and the Eastern Cape Provinces (Shephard et al., 2005; Adekoya et al., 2018). Fumonisin B_1 (FB_1) was the predominant mycotoxin detected in all beverage samples (38-1066 µg/L) (Shephard et al., 2005:9634; Katongole, 2008:22; Adekoya et al., 2018:23).

Traditional beverage are not only produced for enjoyment but also has a high nutritional content. Umqombothi is known to provide the recommended requirement of thiamine and riboflavin (B vitamins) and 40% of the recommended daily requirement of niacin (Lues et al., 2008:164; Konfo et al., 2015:191; Adekoya et al., 2018:22; Hlangwani et al., 2020:7). Optimisation of the brewing process in terms of (i) fermentation time and temperatures, (ii) stability , (iii) sensory characteristics, (iv) the implementation of quality control measures will significantly improve the shelf life of Umqombothi. Hygiene training and the implementation of regulations governing the licensing of informal brewers will further improve the maintenance of general hygiene conditions during production. These improvements could minimize the health risks posed by traditionally prepared indigenous beverage.

2.10 Traditional uses of Umqombothi

Umqombothi plays a significant role in cultural, social, and spiritual activities in communities (Dancause et al., 2010:1124; Hlangwani et al., 2020:2). It is used to celebrate the homecoming of young men, known as abakwetha in the Xhosa culture, after initiation and ritual circumcision. It also forms part of the process of contacting the ancestors (known as amadlozi) and plays a central role in many celebrations or life events such as weddings, funerals, and traditional meetings (imbizos) (Katongole, 2008:60; Dancause et al., 2010:1124; Hlangwani et al., 2020:2). A particular type of firewood, ulathile, should not be used during brewing, as it is believed that when people are drinking Umqombothi, there would be conflicts.

2.11 Different types of beer

Beer is considered a form of art and expression of the human spirit, and technical sciences are used as a tool to create it, and psychology to market and sell it. The combination of numerous factors creates a consistent combination of beer characteristics The type of ingredients result to the development of different beer styles worldwide (Eaton, 2006:77; Parker & Bri, 2012:134). Approximately 40000 different beers are for sale around the world.

Individual creation and culture surrounding their enjoyment and celebration assist in defining each beer's uniqueness because many beers are similar in style (Papazian, 2006:41). There are approximately 100 different beer styles originating from America, Belgium, German, Irish and Japan and most of them are available in the South African beer market. Many beer styles remain catalogued and famous in their regions (Papazian, 2006:41). Umqombothi is a traditional South African beverage that is only accessible in South Africa due to its short shelf life and lack of standardization. Traditional spontaneous fermented beer, simirla to Umqombothi beverage have been investigated and standardised all over the world, and the traditional has been conserved. Four styles of classic beer survived over the years, i.e., Lager, Porter, Stout, and Blond Ale. Table 2.3 illustrate different types of beer around the world.

Table 2.3: Different types of beers around the world

Beer styles	Difinition
Lambic beer	Belgium Lambic beer, a sour beer brewed from 70% malted barley and 30% wheat, is the earliest known style. It is accomplished by the natural fermentation of bacteria and yeast (Hornsey & Brewery, 2016:349). The spontaneous fermentation process of up to three years results in a Lambic beer (Spitaels et al., 2015:23).
Weissbier	Weissbier has a pale amber colour and is highly carbonated and the alcohol content (4.5-6%) makes it a medium-body beer. Because yeast is present, it has a fruity, nutmeg-like, and yeast-like flavour (Papazian, 2006:65). Weissbier, often known as Hefeweizen, is a turbid beer prepared with barley grits and 50% wheat malt.
Stout beer	Stout is a dark ale whose origin lies in British porter beer, and it is the first style of beer in brewed in Britain. The current style of stout emerges from the descriptive use of the word stout to describe a fuller-flavoured and robust version of porter. Style of stout is brewed with the addition of the distinctive character of malted roasted barley (Papazian, 2006:49)

2.12 Common processing to obtain Umqombothi.

Fermented cereal beverages are produced through an extended process that requires careful monitoring. Various steps are followed to get to the final product, and the initial step is manually mixing the ingredients (malted or unmalted) with water. Umqombothi beverage are brewed with pigmented sorghum varieties (red or brown). The white varieties are always mixed with red sorghum because consumers prefer to drink coloured beverage, which they believe are healthy. The next step is fermenting the mixture at room temperature (sometimes at a controlled temperature) for 24 hours. The product separates typically into two phases, water on top and particles at the bottom. The water will be boiled, the slurry will be added during boiling, and the product will be cooked for ±1 hour. A common process flow chart for obtaining Umqombothi is presented in Figure 2.3.

The product will then be cooled overnight or a few hours before (Katongole, 2008: 65) inoculating it with the previous day's brew (back slope) or prepared starter culture using sorghum or Mnanti beer powder. The product will then be fermented for the second time at room temperature (sometimes at a controlled temperature) for 24 hours. The product will then be sieved to obtain the final product. Sieving can be done using a different sieve, but the sieve must not remove all the residue as it is one of the Umqombothi characteristics. The beverage have a relatively low alcohol content (2-4.5% v/v), a pH of 3.3-4. Their colour varies from pale buff to pink-brown according to the ingredients used, and they are consumed in an actively fermenting state. The shelf life is relatively short (2-3 days).

2.13 Previous research on sorghum beer

Production of Umqombothi involves the extraction of the nutrient rich liquid from the cereal through fermentation. This section will focus on different methods used by researchers to prepare Umqombothi. Table 2.4 summarizes different fermentation methods employed to obtain traditional cereal alcoholic beverages. Umqombothi has be prepared using sorghum malt and maize meal by method reported by (Schaepdrijver, 2004:592). Grain immersed in flowing water for 12 days (sorghum) or up to 4 days (maize), with lengthier periods employed when the weather was chilly. Grain was sprouted for 25 days, still in the sack or in a container, until shoot development was considered satisfactory (1.9 cm, (0.75 in.) for sorghum, 1.3 cm, (0.5 in.) for maize). The malt was typically dried in the sun or within a hut, but it was also consumed uncooked. During the first day, unground grain or (preferably) a 50:50 blend of grain and malt was steeped in water. In the morning, after draining, the wet grain was finely mashed to a paste between stones, and the dough was formed into lumps. In the afternoon, the paste was simply covered with boiling water in a pot, and cold water was added to regulate the temperature based on the brewster's discretion. As the mixture cooled gradually, spontaneous acidification occurred.

The water gathered from above the dough was cooked with more water the next day, while the dough was combined with new boiling water to make a thin porridge that was added to the boiling water. The liquid had thickened because the starch included had gelatinized after a boil of 20-40 minutes (longer times were required for maize, 60 minutes). The mixture was far too thick to be poured with a spoon. Most of the mixture was allowed to cool rather slowly, but a tiny portion was chilled fast and was combined with powdered malt, whereupon starch conversion and a spontaneous fermentation occurred, generating a 'starting culture'.

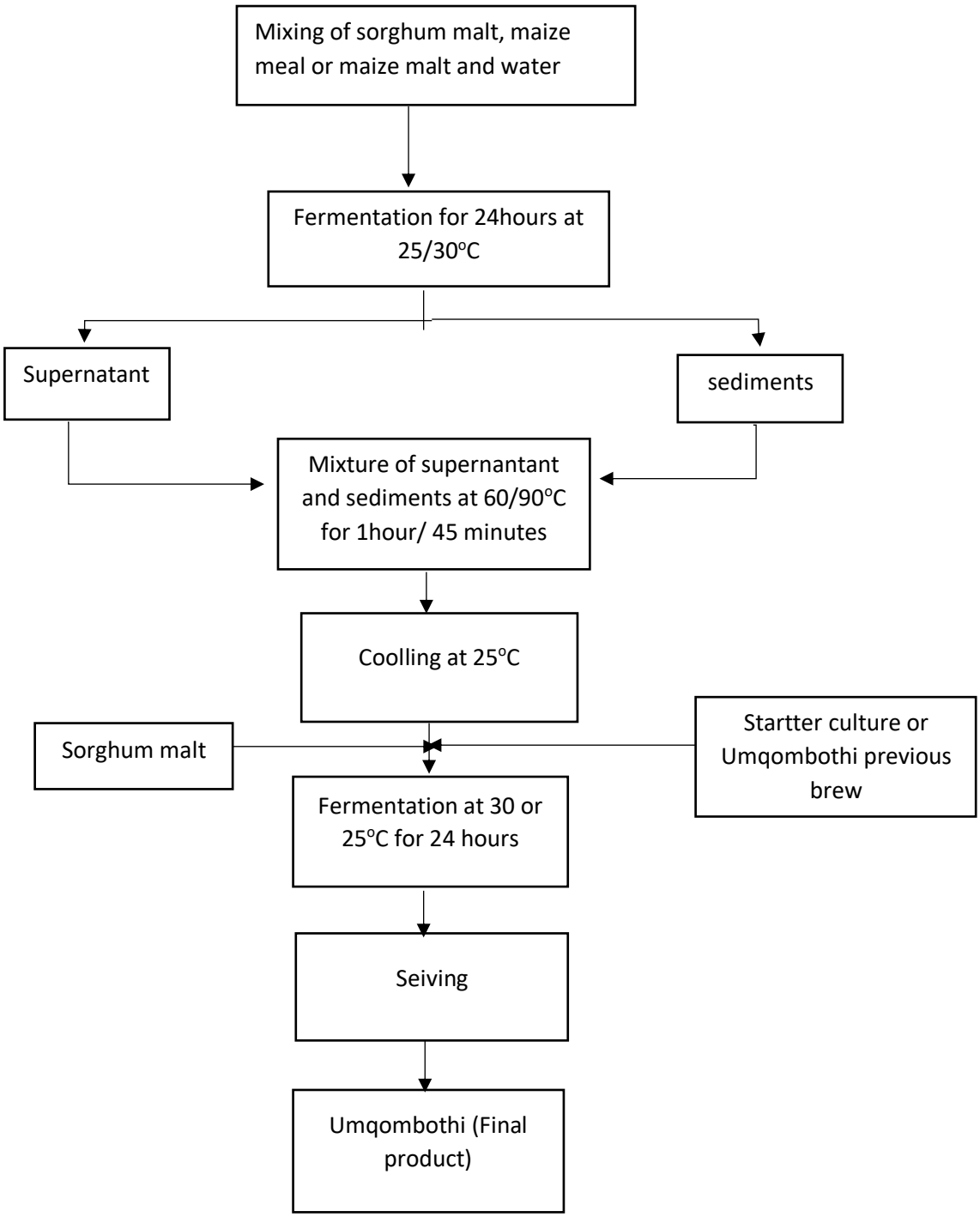

Figure 2.3: Common process flow chart to obtain Umqombothi

Table 2.4: The different method reported for production of Umqombothi

Umqombothi beverage	Ingredients Preparation	Inoculum	Reference
Katongole (Umqombothi)	Unmilled (sorghum malt and maize meal)	Backslop (previous Umqombothi)	(Katongole, 2008:61)
Hlangwani (Umqombothi)	Unmiilled (sorghum malt and maize meal)	Backslop (previous Umqombothi)	(Hlangwani et al., 2021:2)
Native beers (Umqombothi)	Unmilled (sorghum malt and maize meal)	Backslop (previous Umqombothi)	(Schaepdrijver, 2004:592)
Hlangwani (Umqombothi)	Unmilled (sorghum malt and maize meal)	Backslop (previous Umqombothi)	(Hlangwani, et al., 2021:2)

When the main mash had cooled sufficiently, more ground malt was added, in an amount that exceeded the initial amount of grain by about 25%, together with the fermenting'starter culture,' resulting in a speedy initiation of fermentation. After the fermenting process was complete, the mixture was filtered through a woven grass strainer. The filtered liquid continued to ferment, while the strainings were saved for manufacturing a'small beer'. Before the beer was drank, it fermented for another 12 days. Umqombothi prepared by a method reported by Katongole, (2008:61). Maize flour (1 kg) inoculated with a hand-full of sorghum malt and soaked in water for 24 hours. The mixture was then cooked into a soft porridge and allowed to stand for 6 h to cool to room temperature. Additional sorghum malt (0.25 kg) was then added to the porridge and thoroughly stirred. This was then inoculated with a small portion of o umqombothi (20 milliliters) from a previous batch. The mixture was then left to ferment for 18 hours and then strained through a sieve with a pore size of ca. 0.5 mm. The chaff was sun-dried and set aside for use as inoculum in later productions. Nine batches of umqombothi were prepared this way using the same batch of materials.

Hlangwani et al., (2021:2) describe the preparation of Umqombothi from sorghum malt and maize meal. In a sterile 10 liters bucket filled with 7 l tap water, 500 g of pre-packaged King Korn malted sorghum (Mtombo-Mmela) (Tiger Brands, Bryanston, South Africa) was combined with 1000 g of White Star maize meal (Pioneer Foods, Bryanston, South Africa). The mixture was gently mixed, covered, and incubated at 25^{o}C for 24 hours (Labcon, Chamdor, South Africa). Following that, the soured mixture was gently mixed and cooked for 30 minutes at 45^{o}C to form a typical beer porridge (isdudu). After allowing the porridge to cool to 25^{o}C, 500 g of King Korn malted sorghum (Tiger Brands, Bryanston, South Africa) was added and gently mixed. The combination was then fermented for 24 hours at 30^{o}C (Labcon, Chamdor, South Africa). The final beer was subsequently subjected to physicochemical testing.

The brewing process followed a method described by Hlangwani, et al., (2021:2) whereby In a sterile 10 L bucket filled with 7 l sterile tap water, 1000 g of Korn malted sorghum (Mtombo-Mmela) (Tiger Brands, Johannesburg, South Africa) was combined with 1000 g of White Star maize meal (Pioneer Foods, Paarl, South Africa). The mixture was gently mixed, covered, and left to sour for 24 hours at 25^{o}C. The optimum beer brew (OPB) was made by heating the blended components for 1.1 hours at 95^{o}C before cooling to 25^{o}C. Sample of 500 g of King Korn malted sorghum (Mtombo-Mmela) (Tiger Brands, Johannesburg, South Africa) was added to the chilled porridge and gently mixed in. The combination was then fermented at 29.3^{o}C for 25.9 hours to produce the OPB. The customary brew (CB), on the other hand, was made by heating the blended ingredients for 30 minutes at 95^{o}C and allowing them to cool to 25^{o}C. Sample of 500 g of King Korn malted sorghum (Mtombo-Mmela) (Tiger Brands, Johannesburg, South Africa) was added to the cooled porridge and gently mixed in before being fermented at 25^{o}C for 24 hours. The fermentation duration and temperature settings for CB were led by available research on umqombothi processing, whereas those for OPB were based on an earlier study that optimized fermentation conditions for umqombothi processing. Sample of 500 g of King Korn malted sorghum (Mtombo-Mmela) (Tiger Brands, Johannesburg, South Africa), 1000 g of White Star maize meal (Pioneer Foods, Paarl, South Africa), and 7 l tap water were combined to make the mixed raw ingredients (RI).

2.14 Improvement of the manufacturing process of Umqombothi

Mashing, boiling, souring, and fermentation are the general steps of the Umqombothi production process. Several studies have focused on evaluating these stages, especially mashing, and mixing of ingredients, and spontaneous fermentation of traditional Umqombothi. It was found that the weighing of the ingredients, fermentation time and temperature depend on the judgment of the brewer (Adekoya et al., 2018:22). It was further concluded that a lack of control during the production process negatively affects the shelf life and stability of opaque beverages (Sefa-Dedeh et al., 1999:593; Kutyauripo et al., 2009:271).

2.14.1 Mashing and cooking

Most fermented beverages are produced under uncontrolled conditions in most parts of Africa, using traditional customs (Konfo et al., 2015:190). The short shelf life of this traditional brewing method is affected by the microbial content, presence of toxic compounds, general hygiene practices as well as the knowledge and performance of the informal brewers (Ncube et al., 2020:256). The shelf life is limited to a day or two after production, therefore the availability of this beverage is limited (Tokpohozin et al., 2016:218). It is essential to improve the production process of this product to preserve its nutritional and valuable qualities, while still maintaining quality standards. (Briggs et al., 2004:598). People will continue to brew Umqombothi to earn income and conduct traditional ceremonies as part of their African heritage. Therefore,

optimizing the production process with regard to ingredients used, fermentation parameters, cooking time, and temperature is essential.

Mashing/cooking is performed to extract fermentable sugars, amino acids, and vitamins from the ingredients. Maize meal (500 g), sorghum (100 g), and maize malt (100 g) are mixed with 5 l of warm water. The suspension obtained is homogenized and allowed to stand at 25^{o}C and 30^{o}C for 24 hours for spontaneous fermentation to occur. The mixture separates into two phases, i.e., supernatant and sediments. The water is boiled, and slurries (sediments) are added during boiling. Thereafter, the mixture is cooked at 60^{o}C for 40 minutes. The malt provides sufficient enzymes to generate a well-balanced fermentation medium. During the preparation of Umqombothi, mashing starch is required as a source of sugar and as a thickening and suspending agent (Lyumugabe et al*.,* 2012*:*508*,* Konfo et al*.,* 2015*:*192*).* Characteristics of the creamy body come from the gelatinization of starch and keeping in suspension the particles of grain and malt that are essential constituents of beverage. During the Umqombothi production process, a complete conversion of starch is avoided as this will result in thinning of the beverage (Schaepdrijver, 2004:592). Umqombothi is opaque and have high viscosity due to a small particle of cereal grain, starch granule, and suspended yeast. Umqombothi have high viscosity due to gelatinization and the incomplete breakdown of starch (Schaepdrijver, 2004:592; Konfo et al., 2015:190*).* Boiling the wort is performed for several reasons, mainly to bring about the denaturation of malt enzymes and to sterilize the wort (Lyumugabe et al., 2012:192).

2.14.2 Fermentation

Umqombothi wort is inoculated with a natural yeast culture and fermented overnight at 25^{o}C. The product is further spontaneously fermented at 30^{o}C and 25^{o}C for 24 hours. The inoculum contains a mixed culture of unknown bacteria and yeast. Lactic acid bacteria and *S. cerevisiae* are the predominant organisms (Katongole, 2008**:**8; Adekoya et al., 2019:22). In the case of European beer, the fermentation process is started by a defined starter culture containing specific yeast strains (*S. cerevisiae or S. carlsbergensis*) (Hornsey & Brewery, 2016:352*).* The spontaneous fermentation of sorghum and maize malt wort produces African traditional leaven. Unlike European beer made with barley, Umqombothi is a typical example of lactic fermentation followed by alcoholic fermentation in which initially, LAB and later yeasts play the dominant role (Katongole, 2008:13; Usai et al., 2013:146). Bacteria typically dominate the early stages of fermentation due to their higher growth rate. A symbiotic relationship could explain the simultaneous presence of LAB and yeast. Lactic acid bacteria was responsible for the souring of the product and making the environment favourable to the proliferation of yeasts (Konfo et al., 2015:192; Coulibaly et al., 2020:195; Hlangwani et al., 2020:9).

2.15 Shelf life of Umqombothi

Indigenous African traditional beverage have a short shelf life. The souring induced by ongoing microbial activities after producing and developing off-flavours is a detrimental change that makes the beverage unacceptable (Sefa-Dedeh et al., 1999:594; Kutyauripo et al., 2009:271; Konfo et al., 2015:192; Hlangwani et al., 2020:7). The beverage limited shelf life or stability has been reported as the major problem of Umqombothi (Briggs et al., 2004:590). The beverage is consumed while it is still fermenting, and the wort from which the beverages are made is inoculated with wild yeast and the beverage therefore always contains residual microflora originating mainly from its ingredients (Usai et al., 2013:146; Adekoya et al., 2018:22; Hlangwani et al., 2020:4). The resulting beverage are microbiologically unstable and contaminated with yeasts and bacteria at varying levels. Katongole (2008: 8) isolated the following bacterial genera from Umqombothi: *Leuconostoc*, *Lactobacillus, Streptococcus, Pediococcus, Micrococcus,* and *Bacillus.* The fungal genera are mainly *Aspergillus, Paecilomyces, Cladosporium, Fusarium, Penicillium,* and *Trichothecium,* whereas the most common fermenting yeast genus are *Saccharomyces*, which contributes to alcoholic fermentation (Adekoya et al., 2018:23; Hlangwani et al., 2020:9). Some of these microorganisms can be harmful to consumers' health (Lues et al., 2008:165).

According to Schaepdrijver (2004:592) and Lues et al. (2008:169) the reasons for the short shelf life can be attributed to factors of the production process, and the environmental “sanitary”, storage (high temperatures) and marketing conditions. Studies conducted on many indigenous traditional beverages and European beers have shown that they undergo autolysis at the end of fermentation, and the cell constituents are released into the beverage. Due to the presence of the yeast, the readily available nutrients contaminating microflora increase rapidly in numbers leading to their metabolism to change the desired characters of the beverage to change to undesirable characters (Sefa-Dedeh et al., 1999:593; Lyumugabe et al., 2012:510). Lactic acid bacteria production of unwanted acetic acid and volatile off-flavors is also responsible for the quick spoiling of Umqombothi, rendering the beer's taste, odor, and texture unappealing to the customer. (Briggs et al., 2004:590; Adekoya et al., 2019:1).

Pasteurization increases the shelf life of European industrial beers and certain sorghum beverages, e.g. Chibuku in Zimbabwe, by destroying spoilage microbes (Kutyauripo et al., 2009:271). Unfortunately, this process is not applied in traditional beverage making. When pasteurization at (72-76^{o}C for 12 to 15 minutes) was attempted it led to an unacceptable increase in the beverage viscosity through further gelatinization of the starch adjunct and elimination of amylolytic enzymes and favourable beverage characteristics by destroying the active yeast (Briggs et al., 2004:592). On the other hand, the pasteurization of beverage destroys many yeast cells, making the B-group vitamins more available to consumers (Lyumugabe et al., 2012:272). Stability in the *tchoukoutou* beer was obtained for at least 6 months after bottle fermentation was stopped by pasteurization in a water bath at 75-80^{o}C for

15 min. The shelf life of Tugela gold sorghum beer was extended to a comparable level to that of European barley beers through pasteurization (Lyumugabe et al., 2012:512; Konfo et al., 2015:192).

2.16 Future perspectives

Starter cultures are increasingly being employed to optimize and manage the production process, particularly the fermentation steps. However, most of these starter cultures were introduced during the fermentation. To solve this problem, the use of pasteurization coupled with carbonation could provide an effective alternative. Starch gelatinization of maize takes place at 62–74^{o}C, and of sorghum at 69–75^{o}C. Because the acid pH value during the cooking process inhibits the activity of sorghum amylase, maize and sorghum granules should be heated and mashed at 60^{o}C as their gelatinisation temperature is much higher (Yeo & S. Q. Liu, 2014:1613; Motlhaodi et al., 2018:123). Umqombothi typically contains gelatinized and ungelatinized granules. The pasteurization treatment will affect the sensory properties of the beverage, and heat will gelatinize the ungelatinized granules in the beverage and make it more viscous (Briggs et al., 2004:598). According to Briggs et al. (2004:598), Umqombothi flash pasteurization is more successful at 75-80^{o}C for 20-25 seconds. According to Schaepdrijver, (1998: 590), Umqombothi can be flat because of the product's high solid content, which prevents proper carbonation. Beverage with additional yeast and a calculated amount of sugar could only be pasteurized in bottles after the yeast has fermented for 5-12 days and before the carbon dioxide pressure reaches 20-30 psi. The beverage was relatively stable, acceptable, and had an unusual flavour. Elimination of the second edition of sorghum malt in the brewing process can reduce the bacterial load and reduce the rate and amount of acid form (Kutyauripo et al., 2009:272).

2.17 The future of Umqombothi

According to the Food and Agriculture Organization Corporate Statistical Database (FAOSTAT) that is currently available for 2017, Africa is the region that contributes the most to global sorghum production, with an estimated 29.7 million tonnes produced. Sorghum grains are highly adaptable and are typically grown in tropical, subtropical, and temperate climates (Sawadogo-Lingani et al., 2021:6). Due to its drought tolerance, climate intelligence and nutritional content, this crop is widely used in a variety of industries, including animal feed, biofuels, forage, ethanol production, and the preservation of fodder. It continues to be one of Africa's most adaptable food crops (Kewuyemi et al., 2022:1528). Sorghum, rice, maize, and millet are a few examples of cereals that do not contain gluten, the protein portion of wheat. Customers with coeliac disease, an immune-mediated enteropathy of the small intestine that develops in people who are genetically susceptible to it, have the highest demand for gluten-free products (Yeo & Liu, 2014:1612).

South African authorities have raised concerns regarding the occurrence of microorganisms and the presence of toxic compounds in Umqombothi, as well as the general hygienic conditions during preparation (Lues et al., 2008:165). According to Adekoya et al. (2018:23) Umqombothi is brewed in an environment conducive to fungal colonization and together with the use of low-grade cereals, contamination with mycotoxins cannot be ruled out. People will continue to brew Umqombothi for traditional ceremonies, as part of their African heritage, and to earn an income. Several studies on different sorghum beverage in Africa have shown that sorghum beverage are not just produced for enjoyment, but also has nutritional benefits. To constantly produce high-quality Umqombothi beverage, the application of a defined starter culture is strongly recommended as well as a controlled fermentation process. The manufacture of Umqombothi is vital not only for mental as well as social delight, but also for the country's food system, economics, and history.

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CHAPTER 3

PHYSICOCHEMICAL AND MICROBIOLOGICAL CHANGES DURING TWO-STAGE FERMENTATION PRODUCTION OF UMQOMBOTHI

Abstract

Umqombothi is a traditional South African fermented beverage. The brewing process limits its consumption to a day or two after production due to the constant production of carbon dioxide. In this study the physicochemical and microbial changes in Umqombothi produced at two-stage fermentation temperatures [U1 (30-30^{o}C), U2 (30-25^{o}C), U3 (25-30^{o}C)] were studied over 52 hours. Samples were collected before first fermentation (BFF), after first fermentation (AFF), before second fermentation (BSF), after second fermentation (ASF) and after the final product (FP). For all three fermentation temperatures, there was a significant increase (p < 0.05) in microbial counts and a significant drop in pH following fermentation stages (AFF and ASF), with a considerable decrease in total soluble solids (TSS) after ASF. The total viable count (TVC), lactic acid bacteria (LAB), yeast, and mould were not detected in the BSF samples for all three fermentation temperatures. The LAB count was significantly (p < 0.05) different at 5.18, 5.36 and 5.25 log CFU/mL for U1, U2 and U3, respectively. The pH was 3.96, 4.12 and 4.34 for U1, U2 and U3, respectively, and was significantly (p < 0.05) different. Total soluble solids significantly (p < 0.05) increased at the BSF at all temperatures. There was no significant (p > 0.05) difference in specific gravity and ethanol content of Umqombothi at all fermentation temperatures. At all fermentation temperatures, Umqombothi was characterised by redness and yellowness, with that collected from U1 being the lightest in colour (L* = 71.24). Colour difference (ΔE) in the range of 4-8 was perceivable but acceptable as they had a ΔE value of 3.58, 2.07 and 2.02 for U1–U2, U1–U3 and U2–U3 respectively. Umqombothi produced at 30^{o}C for first and second fermentation (U1) was the most preferred by the consumer panellist and consequently, the best fermentation temperature to produce Umqombothi.

3.1 Introduction

Hundreds of years before handcrafted artisan beverages became fashionable, Africans brewed homemade beverages from ingredients that were locally available. Indigenous fermentation methods in Africa are, at best, an art form, with quality varying according to the kind and quality of raw materials employed, ambient conditions, and containers used. (Simatende et al., 2015:120; Adekoya et al., 2018: 21; Aka et al., 2020:1; Ncube et al., 2020:256). Umqombothi remains one of the oldest and most popular traditional beverage among black South Africans. The ingredients are locally available, and it is produced by local

farmers. Umqombothi also plays a fundamental role in traditional brewing history, people's culture and beliefs (Lyumugabe et al., 2012:510; Usai et al., 2013:146; Hesse, 2015:94)

Umqombothi is a B vitamin-rich beverage (Schaepdrijver, 2004: 598; Lyumugabe et al., 2012:510; Adekoya et al., 2018:22; Hlangwani et al., 2020:7) brewed mostly by women either for marketing purposes, or to be consumed at social gatherings, weddings, or ritual events (Dancause et al., 2010:1124; Briggs et al., 2004:589). The colour varies from pale buff to pink-brown to cream-colour after sieving, and has a flavour similar to yoghurt (Katongole, 2008: 13). It has a short shelf life of 2-3 days, alcoholic content of 2-3% pH of 3.3-4.2 and 0.02-0.26% lactic acid content (Nicholas, 2008:13; Adekoya et al., 2018:22). The beverage should be consumed in its active state of fermentation since the composition constantly changes due to the frequent production of carbon dioxide (Usai et al., 2013:146; Hlangwani et al., 2020:5), while the development of acetic acid results in an acidic and poor flavour. Lactic acid bacteria (LAB) and yeast are the predominant microorganisms involved during fermentation (Agu & Palmer, 1998:254; Simatende et al., 2015:120). Lactic acid bacteria are mainly responsible for souring the product, while yeast is necessary for alcohol production. The art of brewing lies within the challenge to produce enough nutrients for LAB and yeast to proliferate, but too much degradation of the starch is not allowed, as it could cause the beverage to be thin. The production process involves fermentation or souring, boiling, followed by a second fermentation under ambient conditions (Konfo et al., 2015:192; Hlangwani et al., 2020:9). The beverage is commonly unstable, and thus its organoleptic traits fluctuate. In addition, the traditional Umqombothi fermentation process is carried out under uncontrolled conditions (e.g., uncontrolled temperature and a non-sterile environment). The challenge lies in consistently producing high quality beer with a long shelf life (Lyumugabe et al., 2012:524; Konfo et al., 2015:192).

Umqombothi's production process has not changed from its usual fermentation at ambient temperatures, cooking at unstandardized temperatures and time, and pitching a back slope culture containing unknown bacterial strains, from a previous overnight brew. The whole process is dependent on the brewer's judgment (Konfo et al., 2015:192; Hlangwani et al., 2020:2). The production process mainly lacks controlled hygienic practices as well as a standardised method. The effective determination of chemical and microbiological content and nutritional composition by Hlangwani, (2021:2) encourages the need to investigate the production of Umqombothi (Xhosa version) under different controlled fermentation temperatures. The objective of this study was to determine the optimal fermentation temperatures of Umqombothi and to evaluate the physicochemical, microbiological and sensory characteristics of the final product.

3.2 Materials and methods

3.2.1 Source of material and equipment

Maize meal, sorghum malt, maize malt, and Mnanti beer powder were obtained from a local supermarket in Bellville, South Africa. Maize malt (Umthombo wombona) was purchased from Boxer Supermarket, Eastern Cape Province (Mdatsane), South Africa. Plate Count Agar ("PCA", NCM0010A, Neogen culture media, Lasec South Africa), De Man Rogosa and Sharpe ("MRS", NCM0190A, Neogen culture media, Lasec South Africa), Violet Red Bile Agar ("VRBA", biolab, Merck South Africa) and Rose-Bengal Chloramphenicol Agar ("RBC", NCM0135A, Neogen culture media, Lasec) were obtained from Merck (Cape Town, South Africa). All brewing equipment used in Umqombothi production was acquired from the Department of Food Science and Technology, Cape Peninsula University of Technology (CPUT), South Africa.

3.2.2 Umqombothi production

Figure 3.1 is an illustration of Umqombothi production steps, followed by physical, chemical, microbiological and sensory evaluation. Umqombothi production process is presented in Figure 3.2. Three batches of Umqombothi were produced by weighing out 500 g of maize meal, 100 g of sorghum and 100 g of maize malt in three separate containers labelled U1, U2 and U3. Lukewarm warm water (5 l) was added to each container and mixed to form a homogenous mixture. The three batches were incubated for 24 hours, with U1 at 30^{o}C, U2 at 30^{o}C and U3 at 25^{o}C for the first fermentation to occur naturally. After 24 hours, the three batches were individually separated into two phases, namely the supernatant and the sediment. The supernatant (liquid) of the respective batches was decanted into separate stainless-steel pots and heated to boiling and sediment (solid particles) from each batch was added to the boiling liquid. The temperature was then reduced to 60^{o}C while stirring for 40 minutes until a porridge was formed. The porridge was cooled down to room temperature, and 300 g of sorghum malt and 75 g of beer powder in 250 ml water were used as an inoculum (the mixture of beer powder and water was incubated at 25^{o}C for 24 hours before being added). The three batches were incubated for 24 hours, with U1 at 30°C, U2 at 25^{o}C and U3 at 30^{o}C. This incubation was referred to as the second fermentation. Thereafter, the products were removed from the incubation chamber and strained through a sieve with a pore size of ca 0.55 mm. The supernatant of each batch, referred to as Umqombothi, was transferred to a labelled sterile container while the sediment was discarded.

Triplicate samples for each batch were collected before and after the first fermentation, after cooking, after the second fermentation and after the sieving process. The samples were collected using a sterile sampling cup and stored at refrigeration temperature (4-6^{o}C) before analysis. A sensory evaluation was conducted on the final product samples for each batch.

The samples were evaluated for physicochemical, chemical, microbiological and sensory analysis (Hlangwani, et al., 2021:2).

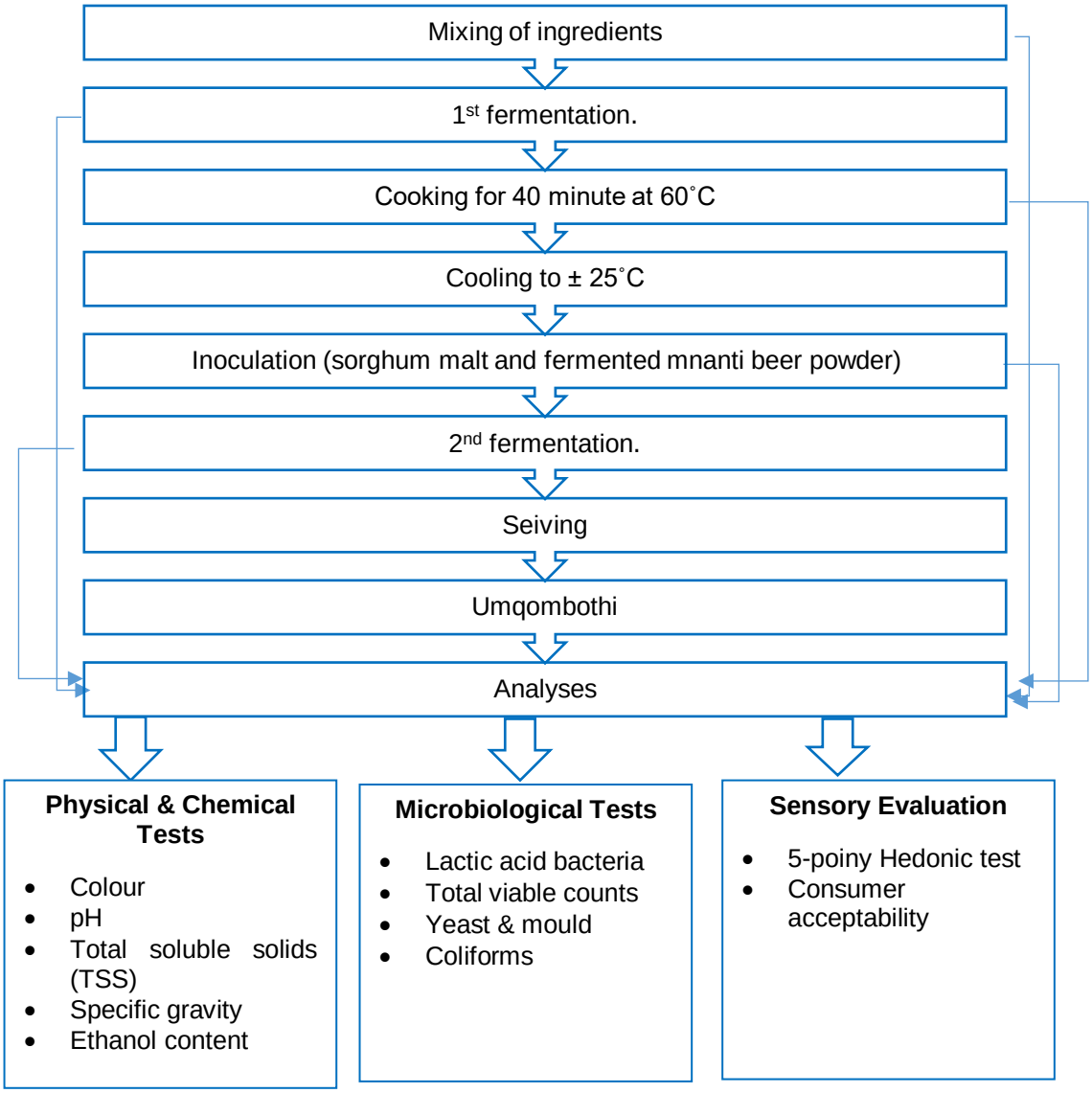

Figure 3.1: Outline of the main Umqombothi production steps followed by physical, chemical, microbiological, and sensory evaluation

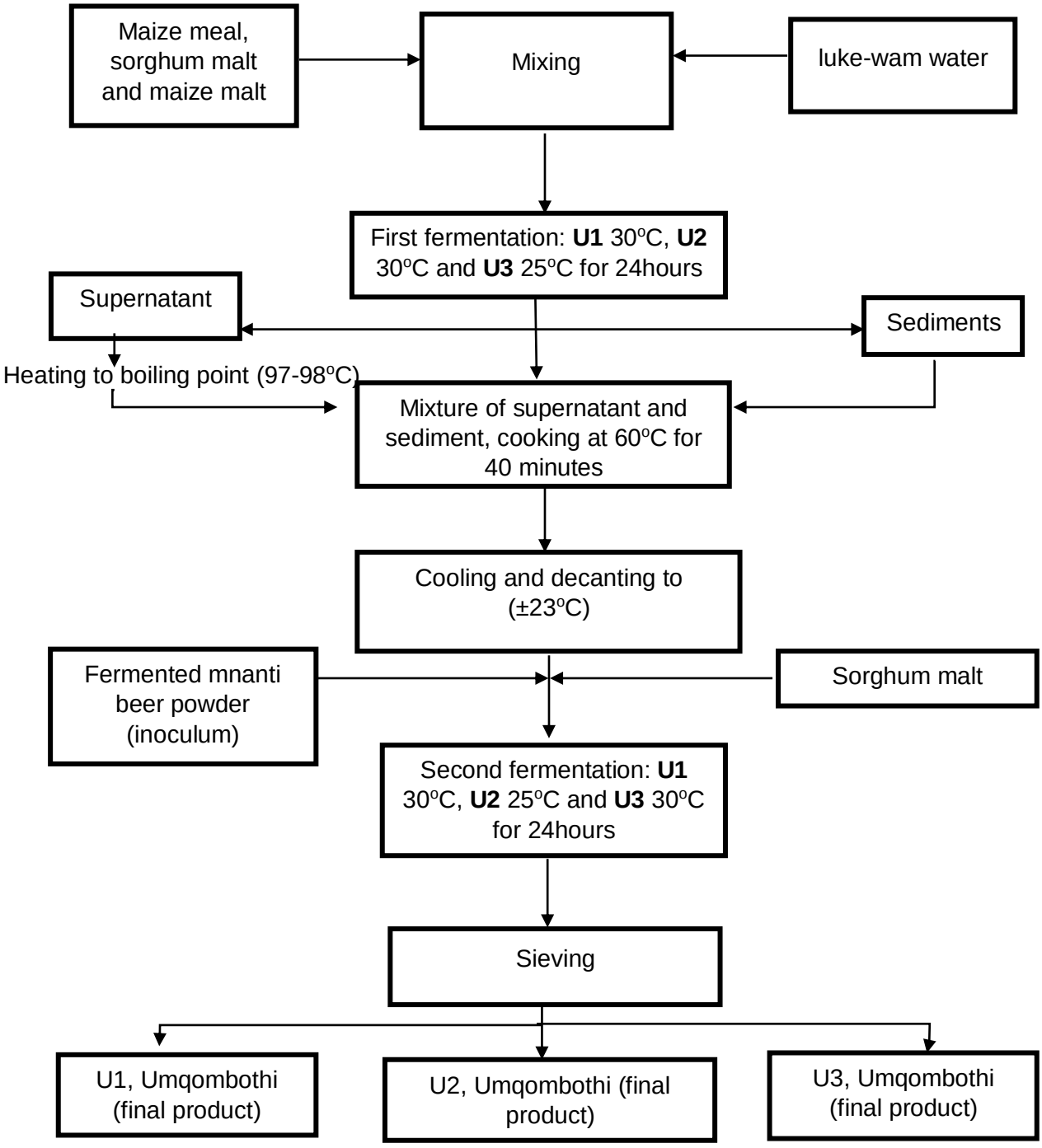

Figure 3.2: Detailed flow diagram of the Umqombothi production process

3.3 Physicochemical analysis

3.3.1 pH

The pH for each sample was measured at ambient temperature (23 ± 2^{o}C) using a calibrated pH meter from Mettler-Toledo GmbH Switzerland (FiveEasy F20). Serial no: B918615210. The glass electrode was calibrated with buffers 7 and 4 (Merck) (Attchelouwa et al., 2017:2).

3.3.2 Total soluble solids (TSS) and Specific gravity

Total soluble solids was measured with a refractometer (Bellingham & Stanley, serial no 036906, UK, 0 -50% °Brix). Specific gravity was measured using a Brew craft refractometer [Portable °Brix & Beer wort specific gravity refractometer, handheld (°Brix 0-32, and Gravity 1.000-1.130)] (Attchelouwa et al., 2017:2). Several drops of the sample were placed on the prism surface. The liquid on the prism must be free of bubbles or floating particles of pulp or other matter. The prism was then closed. To get a valid reading, the instrument was turned towards the light. If necessary, the eyepiece was focused until a clear image appears. The position at which the demarcation line and dark regions cross the vertical scale, is the value of the percentage of total soluble solids reading.

3.3.3 Ethanol

Ten millilitres of each sample were centrifuged at 11000 rpm for 10 minutes in an Avanti J-E Centrifuge (Beckman culture, USA), Serial no: JSE11B30 and cat no: 369003. The supernatants acquired from each sample were used for alcohol content determination by gas chromatography (GC) according to the methods of Katongole (2008:62) (Gas Chromatograph system 7890A, Agilent Technologies, made in China, serial no CN90352528, G 2614A).

3.4 Physical characteristics of Umqombothi

3.4.1 Colour

The colour of Umqombothi was evaluated using a Konica Minolta Spectrometer CM – 5, [Norich (pty) Ltd (Japan)], serial no:1101313, 45°/0° standards, set at standard observer 10° and D65. The instrument was zero calibrated using black (L*=5.49, a*=7.08, b*4.66) and white (L*= 93.41, a*=1.18, b*= 0.75) tiles. Umqombothi 3 ml/3 g was deposited in a light-coloured sample holder, and the reflections were measured on the L*a*b* and LCh colour scales. The L* coordinate is lightness, where a value of 100 represents whiteness, and 0 is a representation of blackness. Coordinate a* referred to the green(-)/red(+) chromatic and coordinate b* referred to the blue(-)/yellow(+) chromatic. Measurements for each sample were performed in triplicate. C* (Chroma) and h (hue) angle 0°=+a*, 90=+b*, 180=-a* and 270°=-b*) was determined as described by Hardy & Jideani, (2018:4).

3.5 Microbiological analysis

Enumeration of the total viable count (TVC), LAB, Coliforms, yeast and moulds in Umqombothi were performed according to the methods described by SANAS 4833:2007, ISO: 4833:2007. The pour plate method was employed for the enumeration of microorganisms in Umqombothi. Counting of all typical colonies was performed using a ColonyStar colony counter from Funke GERBER labortechnik (Berlin, Germany), serial no:8500-6107 and control (Positive controls for VRBA, MRS, RBC, and SPCA performed by streaking with *Escherichi. coli*, *Lactobacillus gasseri*, and yeast (Saccharomyces *cerevisiae*), mould (*Aspergillus*). Controls for ringer, stomacher bags and pipette tips were carried out with 1 ml of ringer into a Petri dish plate (ringers' control), stomacher bag (control), and pipette tips (control) and poured with PCA and incubated at 37^{o}C for 24 hours. Experiments were performed in triplicates. Only plates containing colonies from 30-300 were counted (Hardy & Jideani, 2019:5).

3.5.1 Enumeration of bacteria

Umqombothi (10 g) was weighed into 90 mL sterile Ringer's solution to obtain a 10^{-1} dilution and mixed well. A series of dilutions (10^{-1} to 10^{-6}) were subsequently prepared by transferring 1 ml of a dilution into 9 ml sterile Ringer's solution. For each dilution 1 ml aliquot was thereafter aseptically transferred to a sterile Petri dish. Approximately 12-15 ml of pre-cooled plate count agar (PCA), de Man Rogosa and Sharpe Agar (MRS) and violet-red bile agar (VRBA) were poured in the respective Petri-dishes and carefully swirled to mix well, for the enumeration of total viable bacteria, total lactic acid bacteria and coliforms. Once all plates were allowed to solidify, they were incubated in an inverted position at 37^{o}C for 48 hours (Katongole, 2008:62).

3.5.2 Enumeration of yeast and moulds

From the series of dilutions in 3.5.1 ml aliquot was thereafter aseptically transferred to pre-labelled sterile petri dish and approximately 12–15 ml of pre-cooled Rose Bengal chloramphenicol agar (RBC) was poured into each petri dish plate and swirled to mix well. Once solidified, plates were incubated at 25^{o}C for 5 days in an inverted position (Katongole, 2008:63).

3.6 Sensory evaluation

Sensory evaluation was conducted based on the IFST Guidelines for Ethical and Professional Practices for the Sensory Analysis of Foods. To reduce risks to participants, Umqombothi was assessed for potential microbial, chemical, and physical hazards and certified safe before conducting the sensory evaluation. Ingredients and manufacturing process are similar to what commercial Umqombothi is made. Research activities were restricted to those detailed in the research proposal. Test participants were volunteers, and their right to withdraw from the research was explained to them; informed consent was obtained from research participants, the product ingredient and alcohol content was explained to participants and their anonymity and confidentiality protected. Umqombothi is a traditional South African alcoholic beverage with an alcohol concentration of 2-3 %. Because of the dangers of drinking to one's health and that it impairs one's ability to drive. Panellists were limited to one round of tasting, which consisted of 90 ml of Umqombothi or around 1.8 ml of alcohol per individual. Panellists were also told that they are not required to finish the product because the goal was to taste rather than drink.

A consumer sensory analysis was performed on the three batches of Umqombothi using 50 untrained panellists in the sensory laboratory of the Food Science and Technology Department at Cape Peninsula University of Technology. The samples were presented in 50 ml white polystyrene cups placed side by side on a plastic tray. Each sample cup contained 30 ml of the respective final product batches and was identified by a three-digit code and served at room temperature (23 ± 2^{o}C). A cup of water was provided to clear the pallet before and during tastings. The panellists were provided with a score sheet that consisted of three coded samples with a 5-point hedonic scale ranging from 1 = dislike extremely to 5 = like extremely. The panellists were instructed to rate each sample individually on its merit on the five-point hedonic rating scale for appearance, colour, taste, aroma, texture, and overall acceptability (Attchelouwa et al., 2017:3).

3.7 Data analysis

Multivariate analysis of variance (MANOVA) was used to determine the significant differences ($p \leq 0.05$) in attributes among samples. Duncan's multiple range test were used to separate means where a significant difference existed (IBM SPSS version 22, 2021).

3.8 Results and Discussion

3.8.1 Physicochemical changes in Umqombothi

There were five different sampling stages during Umqombothi production. Before first fermentation (BFF), after first fermentation (AFF), before second fermentation (BSF), after second fermentation (ASF) and the final product (FP) (Umqombothi).

The pH obtained during the manufacturing process of Umqombothi is presented in Table 3.1. The pH obtained during all sampling stages showed a significant ($p < 0.05$) different, with ASF and FP sampling stage for U3 were not significantly ($p < 0.05$) different. However, for U2 a significant difference was only observed from BFF until ASF, thereafter, no further significant reduction was observed between ASF and FP. After cooking, a significant ($p < 0.05$) increase in pH for U1, U2 and U3 respectively, was observed (Table 3.1). Similar trends were observed by Katongole (2008: 65) after the cooking stage (BSF) during the fermentation of Umqombothi. After the second fermentation stage, a significant ($p < 0.05$) decrease in pH was observed for U1 and U3, while U2 showed a significant ($p < 0.05$) increase in pH within 24 hours. Thereafter, a further significant ($p < 0.05$) decline in pH was observed for all three batches after the removal of the solid particles. The observed pH values reported in this study are similar to that (5.71 to 3.56, 6.01 to 3.26, and 5.45 to 3.06 for samples from A (house), B (laboratory) and C township) reported by Katongole (2008:65) for Umqombothi produced in homes, laboratories and township environments. The ph values observed in this study are similar to values reported previously for Umqombothi by earlier researcher (Adekoya et al., 2018: 25; Hlangwani et al., 2020: 5).

When comparing the impact of pH as affectd by fermentation temperatures there was a significant ($p < 0.05$) difference between U1, U2 and U3. The U1 Umqombothi batch produced a significantly ($p < 3.96$) lower pH compared to U2 and U3. A higher pH facilitates microbial growth which may affect the product's shelf life and organoleptic properties (Eaton, 2006:85). An increase in lactic acid bacteria during fermentation causes the reduction in pH in Umqombothi by the production of lactic acid and acetic acid (Togo et al., 2002:2; Kutyauripo et al., 2009:271). Furthermore the pH of the product affects enzyme activity and is crucial in the liquefaction and conversion of malt starch into fermentable sugars (Adekoya et al., 2018:25).

Table 3.1: The effect of two-stage fermentation on pH values of Umqombothi

Production stage	pH		
	U1 (30-30°C)	U2 (30-25°C)	U3 (25-30°C)
Before fermentation (BFF)	5.99 ± 0.01[a]	6.00 ± 0.02[a]	6.02 ± 0.01[a]
After fermentation (AFF)	3.33 ± 0.02[b]	3.38 ± 0.02[b]	4.26 ± 0.01[b]
Before second Fermentation (BSF)	3.61 ± 0.01[c]	3.70 ± 0.01[c]	4.50 ± 0.00[c]
After second fermentation (ASF)	3.41 ± 0.01[d]	3.74 ± 0.01[d]	3.40 ±0.01[d]
Final product (FP)	3.45 ± 0.00[e]	3.75 ± 0.00[d]	3.53 ±0.01[e]
Overall fermentation temperatures	3.96 ± 1.06[a]	4.12 ± 0.98[b]	4.34 ± 0.97[c]

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

Additionally, because higher pH encourages microbial growth, drinks with lower pH have a longer shelf life and higher quality than those with higher pH. Base on the current research, it appears that U1 conditions are the most favorable for fermentating Umqombothi as it results in the lowest pH levels.

The total soluble solids as affected by different fermentation temperatures during Umqombothi production is presented in Table 3.2. The TSS increased significantly ($p < 0.05$) from 1.00-1.10°Brix during the first fermentation for U1, U2 and U3. Before the second fermentation, the TSS significantly ($p < 0.05$) increased to 8.00°Brix, 7.50˚Brix and 7.00°Brix for U1, U2 and U3, respectively. This is due to the hydrolysis of starch into fermentable sugars during the cooking process, however, complete gelatinization of starch was not achieved. Starch in African beverages acts as a source of sugar, a thickener and a suspending agent (Lyumugabe et al., 2012:513). Gelatinization and incomplete degradation of starch cause African beverages to be high in viscosity (Lyumugabe et al., 2012:513). Umqombothi is cooked at 60°C for 40 minutes, below the gelatinisation of sorghum and maize starch which is between 67–81°C and 63–67°C, respectively (Lyumugabe et al., 2012:513;Yeo & Liu, 2014:1613). For the enzymes to gain access to starch molecules, gelatinisation must occur, where starch granules are broken down into fermentable sugars (Russel, 2003:11). During the second fermentation, there was a significant increase ($p < 0.05$) of TSS from 8.00-10.00°Brix, 7.50-10.00°Brix and 7.00-10.00°Brix for U1, U2 and U3. Better access to the substrates (starch, protein and lipids), high water activity and surface area are the ideal conditions for enzymes to function optimally (Willaert, 2006:442).

Table 3.2: The effect of two-stage fermentation on total soluble solids values of Umqombothi

Production stage	Total soluble solids (°Brix)		
	U1 (30-30°C)	U2 (30-25°C)	U3 (25-30°C)
Before first fermentation (BFF)	1.00 ± 0.00[a]	1.00 ± 0.00[a]	1.00 ± 0.00[a]
After first fermentation (AFF)	1.10 ± 0.00[b]	1.10 ± 0.00[b]	1.10 ± 0.00[b]
Before second fermentation (BSF)	8.00 ± 0.00[c]	7.50 ± 0.00[c]	7.00 ± 0.00[c]
After second fermentation (ASF)	10.00 ± 0.00[a]	10.10 ± 0.10[d]	10.00 ± 0.00[d]
Final product (FP)	9.87 ± 0.12[d]	10.00 ± 0.00[a]	10.00 ± 0.00[a]
Overall fermentation temperatures	5.99 ± 4.24[a]	5.94 ± 4.24[b]	5.82 ± 4.18[c]

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letters are significantly ($p \leq 0.05$) different

The TSS obtained in the final product was significantly ($p < 0.05$) lower for U1 and U2. However, there was no significant change in the TSS for U3. A similar pattern in the decrease in the TSS was reported by Kutyauripo et al. (2009:274), by the removal of the second conversion malt. Attchelouwa et al., (2017:5) reported a decrease in TSS from 9.7°Brix to 8.0°Brix on the sample stored between 28°C and 30°C. According to Attchelouwa et al. (2017:5) the TSS act as nutrients used for bacterial growth, as the bacterial numbers increase, the TSS decrease.

When comparing the impact of TSS as affected by fermentation temperatures there was a significantly ($p < 0.05$) different for U1, U2 and U3 respectively. The U2 and U3 Umqombothi batches had a significantly ($p < 10.00$) higher TSS value than U1. However, U1 showed a significant ($p < 0.05$) decrease in TSS as seen by the levelling of solids in the products at the FP sampling stage, not all dissolved solids were available for use by the microorganisms present. It ispossible that the levelling out was due to the inhibitory effect of ethanol. Yeast and LAB use fermentable sugars as a source of food to create ethanol and carbon dioxide (Vriesekoop et al., 2012:339). This is like Tchapalo (Ivorian sorghum beer), which shows a significant ($p < 0.05$) decrease in TSS at ambient temperature (28-30°C) after the first 24 hours from 9.7°Brix to 8.0°Brix (Attchelouwa et al., 2017:5). There was a significant difference ($p < 0.05$) at TSS of Umqombothi as affected by fermentation temperatures. Sample U1 had a significantly ($p < 5.99$) higher TSS than U3 and U2 respectively. Cooking the soured porridge for a suitable period of time is required for starch gelatinization and the release of nutrients bound up in yeast cells (Hlangwani, et al., 2021:4). The cooking duration was discovered to have an effect on the alcohol concentration, TSS, and pH. The breakdown of cooked starch to fermentable sugars by endogenous amylolytic enzymes drives the

development of fermentative microorganisms. The quantity of TSS increases as the endosperm protein surrounding the starch granules softens (during gelatinization), transferring the grain to the retting water (Hlangwani, et al., 2021:4). CThis might explain the rising trend in TSS levels with greater cooking and fermentation time. Total soluble solid will be increased systemically throughout the production stages, and Umqombothi production will be improved with U1 fermentation temperatures.

The ethanol content and specific gravity (SG) of different fermentation temperatures at different sampling stages of the production process are presented in Table 3.3. Beer authenticity can be identified by percentage alcohol content by volume (% ABV) and original gravity, as they are highly indicative parameters. Specific gravity is also a tool to measure the fermentation process (Almonacid et al.,2012). The higher the specific gravity the better the sugar will dissolve in the liquid (wort). The Specific gravity for all sampling stages resulted in no significant difference for batches U1, U2 and U3.

Table 3.3: The effect of two-stage fermentation on specific gravity (SG) values of Umqombothi

	Specific gravity		
Production stage	**U1 (30-30°C)**	**U2 (30-25°C)**	**U3 (25-30°C)**
Before first fermentation (BFF)	1.00 ± 0.00[a]	1.00 ± 0.00[a]	1.00 ± 0.00[a]
After first fermentation (AFF)	1.00 ± 0.00[a]	1.01 ± 0.00[a]	1.01 ± 0.00[a]
Before second fermentation (BSF)	1.04 ± 0.00[b]	1.03 ± 0.00[a]	1.03 ± 0.00[a]
After second fermentation (ASF)	1.04 ± 0.00[b]	1.04 ± 0.00[a]	1.04 ± 0.00[a]
Final product (FP)	1.04 ± 0.00[b]	1.04 ± 0.00[a]	1.04 ± 0.00[a]
Overall fermentation temperatures	1.02 ± 0.02[a]	1.02 ± 0.02[a]	1.02 ± 0.02[a]

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

The original gravity (OG) minus the final gravity (FG) of the wort can approximately provide the percentage alcohol content by volume (% ABV) of the final beverage. Specific gravity can be used to determine the alcohol % of the beer (Spedding, 2016:126; Entwisle et al., 2008:461).

An ethanol content of 2.00% was obtained for all final product samples. The alcohol content (%) reported in this study agrees with the rage of 2 to 3.5% reported by (Hlangwani et al., 2020:6), According to Hlangwani et al. (2020:6), the ethanol content of Umqombothi will differ depending on where and how it was brewed. (Briggs et al. 2004:590) reported alcohol content rage of 1-8%, whereas values of 2.5-4.5% are the most common. Ethanol

characteristics contribute to flavour and impacts the quality of the beer (Vanderhaegen et al., 2007:404). Entwisle et al. (2008:461) noted that microbial activity of conversion of ethanol to acetic acid can cause a decrease in ethanol content as the beer deteriorates. A greater fermentation temperature, in general, impacts the pace of sugar metabolism (i.e., results in a quick increase in alcohol content and other by-products like as volatile compounds) (Hlangwani, et al., 2021:4).

3.8.2 Colour characteristics of Umqombothi

The lightness of Umqombothi from different fermentation temperatures, at different sampling stages, is displayed in Table 3.4. The lightness of sampling stages BFF, AFF, BSF, ASF and FP ranged from 57.11-90.60, 54.51-89.12 and 55.17-91.05 for U1, U2 and U3, respectively. The lightness was significantly ($p < 0.05$) different during all sampling stages of Umqombothi production process for U1, U2 and U3 respectively. The lightness significantly ($p < 0.05$) decreased between BFF and ASF sampling stages during the Umqombothi production process for U1, U2 and U3, respectively. The lightness increased considerably ($p < 0.05$), between ASF and FP for U1, U2 and U3, respectively. This could be due to the removal of solid particles in the final product, increasing lightness as they make the product lighter. Cooking and fermentation are methods for improving the overall safety of the beverage (Hlangwani et al., 2020:10).

Table 3.4: The effect of two-stage fermentation on lightness values of Umqombothi

	Lightness		
sampling stage	**U1 (30-30^{o}C)**	**U2 (30-25^{o}C)**	**U3 (25-30^{o}C)**
Before first fermentation (BFF)	90.60 ± 0.01^{a}	89.12 ± 0.02^{a}	91.05 ± 0.47^{a}
After first fermentation (AFF)	87.02 ± 0.00^{b}	85.02 ± 0.02^{b}	88.80 ± 0.01^{b}
Before second fermentation (BSF)	58.89 ± 0.01^{c}	54.78 ± 0.02^{c}	55.17 ± 0.02^{c}
After second fermentation (ASF)	57.11 ± 0.02^{d}	54.51 ± 0.00^{d}	55.20 ± 0.00^{c}
Final product (FP)	62.59 ± 0.36^{e}	59.66 ± 0.00^{e}	61.68 ± 0.00^{d}
Overall ferementation temperatures	71.24 ± 15.01^{a}	68.62 ± 15.77^{b}	70.38 ± 16.72^{c}

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letters are significantly ($p \leq 0.05$) different

The colour of the wort changes when maize and sorghum malts are introduced as adjuncts in brewing (Puligundla et al., 2021:530). Temperature, polyphenol oxidation, and grist material alter (decrease) the colour of the wort during the processing phases, according to Pahl et al. (2016:113).The values of lightness as affected by fermentation temperatures were 71.24, 68.62, and 70.38 and were significantly ($p < 0.05$) different for U1, U2 and U3 respectively. Umqombothi U1 had a significantly ($p < 62.59$) greater lightness than U3 and U2. However, higher temperature for both fermentation processes exhibited a lightness value of 71.24, which is closer to 100 representing whiteness of the colour scale. Thedecrease and increase of lightness during the production process of Umqombothi, with U1 having higher lightness values than U2 and U3, show that Umqombothi is light in color, as all of the values are close to 70. Seventy is closer to 100 which represent whithness of colour scale.

The redness of Umqombothi obtained from different fermentation temperatures, at different sampling stages is displayed in Table 3.5. According to Rumler et al. (2021:1) the sorghum kernel is initially reddish, so this was expected. The redness of sampling stages BFF, AFF, BSF, ASF and FP ranged from 0.93-8.79, 1.07-7.73 and 0.98-7.97 for U1, U2 and U3 respectively. The level of a* was significantly ($p < 0.05$) different during all sampling stages of the production process for U1, U2 and U3, respectively. The redness significantly ($p < 0.05$) increased between BFF (0.93, 1.07) and FP (6.99, 6.30) fermentation sampling stages for U1 and U2, respectively. The redness significantly ($p < 0.05$) increased in ASF as compared to BSF sampling stages for U1, U2 and U3, respectively. Temperature, polyphenol oxidation, flavonoids, and grist material alter the colour of wort during processing phases, According to Pahl et al. (2016:115) temperature, polyphenol oxidation, flavonoids, and grist material all affect the colour of wort during the processing phases. This could be due to the increase of microbial load during the fermentation stages as well as the effect of heat during cooking. The removal of solid particles significantly ($p < 0.05$) reduced the redness in the final product of all three different fermentation temperatures.

The raw material, cooking, inclusion of sorghum malt and as inoculum after first fermentation could explain the considerable significant ($p < 0.05$) increase of redness during the after fermentation stages. Significantly ($p < 0.05$) increased microbial load during the fermentation stages could explain the increase in the overall effect of redness following fermentation for U1, U2 and U3, respectively. The sieving (removal of solid particles) significantly ($p < 0.05$) decreased the redness in the FP sampling stage for U1, U2 and U3. The redness as affected by fermentation temperatures were 4.01, 3.69, 3.61 and they were significantly ($p < 0.05$) different for U1, U2 and U3 respectively.

Table 3.5: The effect of two-stage fermentation on redness values of Umqombothi

Sampling stage	Redness		
	U1 (30-30°C)	U2 (30-25°C)	U3 (25-30°C)
Before first fermentation (BFF)	0.93 ± 0.01[a]	1.07 ± 0.02[a]	1.03 ± 0.02[a]
After first fermentation (AFF)	1.08 ± 0.00[b]	1.24 ± 0.01[b]	0.98 ± 0.01[b]
Before second fermentation (BSF)	2.26 ± 0.01[c]	2.15 ± 0.05[c]	1.98 ± 0.01[c]
After second fermentation (ASF)	8.79 ± 0.01[d]	7.73 ± 0.00[d]	7.97 ± 0.01[d]
Final product (FP)	6.99 ± 0.01[e]	6.30 ± 0.00[e]	6.11 ± 0.00[e]
Overall fermentation temperatures	4.01 ± 3.37[a]	3.69 ± 2.87[b]	3.61 ± 2.98[c]

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letters are significantly ($p \leq 0.05$) different

The U1 Umqombothi batch had a Significantly ($p < 4.01$) greater redness than U2 and U3 respectively. The yellowness of Umqombothi from different fermentation temperatures, at different sampling stages is displayed in Table 3.6. The yellownes values of Umqombothi were positive, which suggested that Umqombothi is in the yellowness colour space. This is expected because yellow maize is mostly used in traditional beverages (Katongole, 2008:16; Rumler et al., 2021:2). The yellowness of sampling stages of BFF, AFF, BSF, ASF and FP ranges from 4.27-23.15, 4.77-22.35 and 4.77-23.30 for U1, U2 and U3, respectively. The yellowness was significantly different during all sampling stages of Umqombothi production process for U1, U2 and U3, respectively. The yellowness significantly ($p < 0.05$) increased during the sampling stages of U1, BFF (4.27), AFF (5.12), BSF (8.70), ASF (22.73) and FP (23.15), respectively. The yellowness significantly ($p < 0.05$) increased continously from BFF to FP for the U1 production process. The yellowness significantly ($p < 0.05$) increased between BFF (4.77)-ASF (22.35) and decreased for FP (21.32) during the production process for U2. The yellowness significantly ($p < 0.05$) decreased between BFF (5.00)-AFF (4.77) and ASF (23.30)-FP (21.50) during the production process for U3. Fermentation, cooking, and the addition of sorghum malt at BSF had a positive impact on the yellowness of the beverage. The concertation of solids particles in Umqombothi does affect the yellowness, as observed after sieving, when the yellowness significantly ($p < 0.05$) increased for U1 and decrease for U2 and U3, respectively.

Table 3.6: The effect of two-stage fermentation on yellowness values of Umqombothi

	Yellowness		
Sampling stages	**U1 (30-30°C)**	**U2 (30-25°C)**	**U3 (25-30°C)**
Before first fermentation (BFF)	4.27 ± 0.01[a]	4.77 ± 0.02[a]	5.00 ± 0.18[a]
After first fermentation (AFF)	5.12 ± 0.00[b]	5.76 ± 0.01[b]	4.77 ± 0.01[b]
Before second fermentation (BSF)	8.70 ± 0.01[c]	7.20 ± 0.02[c]	7.42 ± 0.01[c]
After second fermentation (ASF)	22.73 ± 0.02[d]	22.35 ± 0.00[d]	23.30 ± 0.01[d]
Final product (FP)	23.15 ± 0.04[e]	21.32 ± 0.00[e]	21.50 ± 0.00[e]
Overall fermentation temperatures	12.79 ± 8.71[a]	12.28 ± 8.12[b]	12.39 ± 8.53[c]

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letters are significantly ($p \leq 0.05$) different

The yellowness values are much higher than the redness values, which further suggests that Umqombothi is dominated by a yellow colour, as it is closer to a hue angle of 90°C, which indicates pure yellowness on the colour scale. The yellowness as affected by fermentation temperatures were 12.79, 12.28, 12.39 for U1, U2 and U3 respectively and they were significantly ($p < 0.05$) different. Umqombothi U1 had a significantly ($p < 23.15$) greater yellowness than U3 and U2, respectively. The constant high fermentation temperatures prove to have a positive impact on the colour of Umqombothi. According to Briggs et al.*(*2004:590) and Katongole (2008:59) the colour of Umqombothi varies from pale buff to pink-brown to cream-colour after sieving, based on the naked eye.

The chroma C* is calculated as $(a^{*2} + b^{*2})^{1}/_{2}$, Chroma is the degree of streght in a colour. Chroma attribute of Umqombothi from different fermentation temperatures, at different sampling stages is displayed in Table 3.7. The chroma of Umqombothi were positive during the production process for U1, U2 and U3. The chroma of sampling stages of BFF, AFF, BSF, ASF and FP ranged from 4.37-24.36, 4.89-23.65 and 4.87-24.63 for U1, U2 and U3, respectively. The chroma was significantly ($p < 0.05$) different during all sampling stages of the production process for U1, U2 and U3, respectively. The vividness significantly ($p < 0.05$) decreased between ASF (24.36, 23.65 and 24.63) and FP (24.19, 22.23 and 22.35) sampling stages during the production for U1, U2 and U3, respectively. This could be due to the sieving (removal of solid particles). The chroma as affected by fermentation temperatures were 13.43, 12.83, 12.93 and they were significantly ($p < 0.05$) different for U1, U2 and U3 respectively. U1 Umqombothi had a significantly ($p < 13.43$) greater chroma than U3 and U2 respectively.

Table 3.7: The effect of two-stage fermentation on chroma values of Umqombothi

	Chroma		
Sampling stage	**U1 (30-30°C)**	**U2 (30-25°C)**	**U3 (25-30°C)**
Before first fermentation (BFF)	4.37 ± 0.01[a]	4.89 ± 0.02[a]	5.10 ± 0.18[a]
After first fermentation (AFF)	5.24 ± 0.00[b]	5.88 ± 0.01[b]	4.87 ± 0.01[b]
Before second fermentation (BSF)	8.99 ± 0.01[c]	7.51 ± 0.04[c]	7.68 ± 0.01[c]
After second fermentation (ASF)	24.36 ± 0.02[d]	23.65 ± 0.00[d]	24.63 ± 0.00[d]
Final product (FP)	24.19 ± 0.05[e]	22.23 ± 0.00[e]	22.35 ± 0.00[e]
Overall fermentation temperatures	13.43 ± 9.31[a]	12.83 ± 8.59[b]	12.93 ± 9.02[c]

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

The hue angle (H°) of Umqombothi from different fermentation temperature, at different sampling stages is displayed in Table 3.8. The hue angle of Umqombothi were positive, during the production process of Umqombothi with U1, U2 and U3, respectively. The hue angle of sampling stages BFF, AFF, BSF, ASF and FP ranges from 68.96-78.11, 70.92-77.82 and 71.15-78.36 for U1, U2 and U3 respectively. The hue angle was significantly ($p < 0.05$) different during all sampling stages of the production process for U1, U2 and U3, respectively. The hue angle significantly ($p < 0.05$) decreased between BFF (77.74, 77.35 and 78.36) and FP (73.18, 73.54 and 74.14) sampling stages during the Umqombothi production process for U1, U2 and U3, respectively. The hue angle increased significantly ($p < 0.05$) AFF and decreased significantly ($p < 0.05$) ASF for U1, U2 and U3 respectively.

The hue angle as affected by fermentation temperatures were 74.68, 74.60 and 75.41 and they were significantly ($p < 0.05$) different for U1, U2 and U3, respectively. Umqombothi U3 had a significantly ($p < 75.41$) greater hue angle than U1 and U2. The increases in the microbial population after fermentation stages could have influence the increased in hue angle. Pahl et al. (2016:113) discovered a similar pattern in which temperature, polyphenol oxidation, and grist material all influenced the colour of the wort during the processing steps.

The color differences (ΔE) of Umqombothi samples fermented at different temperatures ranged from 0.76 to 5.99. Colour difference (ΔE) < 1 is defined as a not noticeable difference, meaning that the observer does not detect the difference. A color difference (ΔE = 1) is defined as a barely discernible change (JND).

Table 3.8: The effect of two-stage fermentation on Hue angle (h°) values of Umqombothi

	Hue		
Sampling stage	**U1 (30-30°C)**	**U2 (30-25°C)**	**U3 (25-30°C)**
Before first fermentation (BFF)	77.74 ± 0.06[a]	77.35 ± 0.16[a]	78.36 ± 0.23[a]
After first fermentation (AFF)	78.11 ± 0.00[b]	77.82 ± 0.13[b]	78.36 ± 0.06[a]
Before second fermentation (BSF)	75.42 ± 0.05[c]	73.38 ± 0.33[c]	75.07 ± 0.06[b]
After second fermentation (ASF)	68.96 ± 0.09[d]	70.92 ± 0.00[d]	71.15 ± 0.00[c]
Final product (FP)	73.18 ± 0.01[e]	73.54 ± 0.00[c]	74.14 ± 0.00[d]
Overall fermentation temperatures	74.68 ± 3.48[a]	74.60 ± 2.71[a]	75.41 ± 2.83[b]

Mean value ± standard deviation of triplicate determination. Mean values in the same followed by different letter are significantly ($p \leq 0.05$) different

Colour difference between 4 and 8 is perceptible but acceptable (Murevanhema & Jideani, 2015:1494), which means that an observer notices the difference and considers it acceptable. The colour difference between Umqombothi samples at the different fermentation temperatures U1, U2 and U3, were perceiveable with ΔE of 3.58, 2.07 and 2.02 for U1–U2, U1–U3 and U2–U3, respectively.

3.8.3 Microbiological population in Umqombothi

The LAB counts during Umqombothi production for different fermentation temperatures are displayed in Table 3.9. The lactic acid bacteria numbers significantly ($p < 0.05$) increased during the first fermentation for U1, U2 and U3, respectively. Hlangwani et al., (2020:9) reported lactic acid bacteria are the most dominant microorganisms during the fermentation of sorghum beer, with fewer occurrences and reports of yeast and fungi which is noticeable in this study.

The environment in which LAB thrives is rich in protein, sugar, vitamins, nucleotides, and lipids typically found in sorghum beverages which therefore explain their predominance in the sorghum microflora (Hlangwani et al., 2020:10). The growth rate can also be linked to their relative superiority in starchy sorghum substrate utilization, as well as veritable carbohydrate metabolisms and strong acid tolerance. Lactic acid bacteria significantly ($p < 0.00$) decreased or were non-detected at the BSF stage for U1, U2 and U3, respectively, which may be altributed to cooking (BSF sampling stage).

Table 3.9: The effect of two-stage fermentation on lactic acid bacteria (LAB) values of Umqombothi

	LAB (cfu/ml)		
Sampling stage	**U1 (30-30^{o}C)**	**U2 (30-25^{o}C)**	**U3 (25-30^{o}C)**
Before first fermentation (BFF)	2.50 ± 0.05^{a}	2.30 ± 0.17^{a}	2.50 ± 0.04^{a}
After First fermentation (AFF)	7.22 ± 0.03^{b}	7.79 ± 0.03^{b}	8.10 ± 0.05^{b}
Before second fermentation (BSF)	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
After second fermentation (ASF)	7.94 ± 0.05^{d}	8.12 ± 0.05^{d}	8.12 ± 0.05^{d}
Final product (FP)	8.24 ± 0.06^{e}	8.06 ± 0.02^{d}	8.06 ± 0.05^{b}
Overall fermentation temperatures	5.18 ± 3.44^{a}	5.25 ± 3.55^{b}	5.36 ± 3.57^{c}

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letters are significantly ($p \leq 0.05$) different

The LAB counts in the overall sampling stages BFF (2.44 log cfu/ml), AFF (7.70 log cfu/ml) and BSF (0.00 log cfu/ml) differed significantly ($p < 0.05$) and there was no significant difference between ASF (8.07 log cfu/ml) and FP (8.12 log cfu/ml). The LAB count significantly ($p < 0.05$) increased in the final product for U1 and decrease significantly ($p < 0.05$) for U2 and U3, respectively. The overall fermentation values of LABs were 5.18 log cfu/ml, 5.25 log cfu/ml and 5.36 log cfu/ml and were significantly ($p < 0.05$) different for U1, U2 and U3 respectively.

The U3 sample had a significantly ($p < 5.36$) higher LAB value than U2 and U3 respectively. The addition of sorghum malt and fermented beer powder as inoculum, before the second fermentation stage (BSF), increased the product's total flora. Previously, LAB in the production of opaque sorghum beverage showed the same trend (Bayoï & Etoa, 2021:77). This category of microorganisms, according to Hlangwani.(2020:7), are exploitative competitors that impede other microorganisms by rapidly utilizing glucose and accumulating acetic and lactic acid. Lactic acid bacteria is one of the most common microorganisms used in sorghum fermentation, being the primary carrier. As the fermentation temperature was reduced to 25^{o}C for the first fermentation, the LAB count for the final product was significantly ($p < 0.05$) decreased to (8.06 log cfu/ml). As noted in a study by Adekoya et al*.* (2018:22) and Katongole (2008:3) , LAB and yeast are the dominant microorganisms in Umqombothi, with the highest LAB counts present at the lowest pH. In the present study, U1 exhibited a LAB count of 8.24 log cfu/ml in the final product at the lowest pH of 3.96 than U2 and U3 (Table 3.1 & Figure 3.3).

The TVC counts during Umqombothi production for different fermentation temperatures are displayed in Table 3.10. The TVC of sampling stages of BFF, AFF, BSF, ASF and FP ranged from 0.00-8.46 log cfu/ml, 0.00-8.19 log cfu/ml and 0.00-8.18 log cfu/ml for U1, U2 and U3, respectively. The TVCs significantly ($p < 0.05$) increased after the fermentation stages for U1, U2 and U3, respectively. The TVC were significantly ($p < 0.05$) increased in the final product for U1, U2 and significantly ($p < 0.05$) decreased for U3. The TVCs significantly ($p < 0.00$) reduced to non-detected at BSF sampling stages for all three fermentation temperatures. The significant ($p < 0.05$) increase in TVCs after second fermentation, could be caused by the addition of sorghum malt and the starter culture. The significant ($p < 0.05$) decrease in TVC in the final product for U1, U2 and U3, could be caused by sieving, with pore size of 0.55mm. There was no significant difference between ASF-FP for U2 and ASF-FP for U3. The overall fermentation temperatures values for TVC were 5.68 log cfu/ml, 5.66 log cfu/ml and 5.86 log cfu/ml.

Table 3.10: The effect of two-stage fermentation on total viable count (TVCs) values of Umqombothi

	Total viable counts (cfu/ml)		
Sampling stage	**U1 (30-30°C)**	**U2 (30-25°C)**	**U3 (25-30°C)**
Before first fermentation (BFF)	4.63 ± 0.06^{a}	4.83 ± 0.04^{a}	4.85 ± 0.02^{a}
After first fermentation (AFF)	7.10 ± 0.06^{b}	7.09 ± 0.02^{b}	8.18 ± 0.03^{b}
Before second fermentation (BSF)	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
After second fermentation (ASF)	8.18 ± 0.01^{d}	8.18 ± 0.07^{d}	8.17 ± 0.14^{b}
Final product (FP)	8.46 ± 0.01^{e}	8.19 ± 0.03^{d}	8.09 ± 0.39^{b}
Overall fermentation temperatures	5.68 ± 3.25^{a}	5.66 ± 3.19^{a}	5.86 ± 3.31^{b}

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

There was no significant difference in TVCs between U1 and U2. U3 exhibited a significantly ($p < 5.86$) higher TVC than U1 and U2. A combination of cooking and fermentation, as performed in the case of Umqombothi, improves the nutrient quality of sorghum and reduces the content of anti-nutritional components to a safe level (Atter et al., 2017:352; Hlangwani et al., 2020:4). The high moisture content, nutrients, and contamination with microorganisms from raw material contribute to the significant increase in TVC in opaque sorghum traditional beverages. The inclusion of tainted sorghum malt after heating would have likely resulted in a

huge increase in the TVC in the BSF and FP sampling stages. The observed TVC counts reported in this study are lower than the 10.8 logs cfu/ml reported by Adekoya et al., (2019:3).

TVC is commonly used as an indicator to evaluate the quality, safety, and shelf-life of food (Hill, 2016:293). The relevant microorganisms are able to grow and survive under aerobic and are considered mesophilic bacteria, as they have a wide growth temperature range of 20-45^{o}C but mainly grow optimally at 35-37^{o}C (Vriesekoop et al., 2012:340). Excessive growth of total aerobes (spoilage bacteria) may lead to spoilage of Umqombothi and reductionof its shelf life, resulting in undesirable sensory characteristics, such as loss of texture, off-flavours and colour (Hill, 2016:293; Adekoya et al., 2018:21). Total plate count colonies, on the other hand, indicate the collective enumeration of all mesophilic bacteria, including lactic acid bacteria, yeast, and mould, as well as total coliforms. The yeast counts during Umqombothi production for different fermentation temperatures are displayed in Table 3.11. Brewing yeast has a direct impact on the character and quality of beer, depending on which type is used to make a particular style. Traditional fermented beverages could be a valuable source of yeast for the brewing business. In addition to *S. cerevisiae*, many traditional fermented beverages and other beverages start spontaneously and frequently contain a mix of wild yeast strains. Yeast is responsible for both the conversion of fermentable carbohydrates into ethanol and the formation of a large variety of flavour active chemicals (Cubillos et al., 2019:386)**.**

The yeast counts of sampling stages of BFF, AFF, BSF, ASF and FP ranged from 0.00-7.56 log cfu/ml, 0.00-7.54 log cfu/ml and 0.00-7.51log cfu.ml for U1, U2 and U3, respectively. The yeast counts of the respective sampling stages differed significantly ($p < 0.05$) for U1 and U3. There was no significant difference in yeast count between FP (3.53 log cfu/ml)-ASF (3.28 log cfu/ml) and FP (3.53 log cfu/ml)-BFF (3.64 log cfu/ml) for U2. The yeast count significantly ($p < 0.05$) increased after the fermentation stages for U1, U2 and U3, respectively. The yeast count significantly ($p < 0.00$) decreased at BSF for U1, U2 and U3, respectively. During the FP sampling stages, sieving (removal of solid particle sizes) led to a significant ($p < 0.05$) decrease in yeast counts for the U1 sample and a significant ($p < 0.05$) increase for the U2 and U3 samples. This removal of solid particles confirmed that the majority of microorganisms are found in the ingredients. The environment in which LAB and yeast grow, according to Hlangwani et al., 2020:4), is rich in protein, sugar, vitamins, nucleotides, and lipids, which could explain their predominance in the sorghum microflora. Umqombothi is nutrient-rich, which explains why yeast numbers increase dramatically after fermentation (Briggs et al., 2004:592; Hlangwani et al., 2020:4; Katongole, 2008:59). Fermentation normally causes enzyme activation, a decrease in pH, and an increase in metabolic and microbial activity.

Table 3.11: The effect of two-stage fermentation on yeast values of Umqombothi

	Yeast (cfu/ml)		
Sampling stage	**U1 (30-30^{o}C)**	**U2 (30-25^{o}C)**	**U3 (25-30^{o}C)**
Before first fermentation (BFF)	3.49 ± 0.03^{a}	3.64 ± 0.08^{ae}	3.67 ± 0.03^{a}
After first fermentation (AFF)	7.56 ± 0.05^{b}	7.54 ± 0.05^{b}	7.51 ± 0.01^{b}
Before second fermentation (BSF)	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
After second fermentation (ASF)	3.68 ± 0.07^{d}	3.28 ± 0.01^{de}	5.37 ± 0.07^{d}
Final product (FP)	3.41 ± 0.04^{e}	3.53 ± 0.29^{eda}	5.75 ± 0.07^{e}
Overall Fermentation temperature	3.63 ± 2.48^{a}	3.60 ± 2.48^{b}	4.46 ± 2.63^{c}

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

This causes the substrate to break down, boosting of the nutritional quality of the beverage, which favour yeast growth (Hlangwani et al., 2020:10). The yeast count in relation to the overall fermentation temperatures were 3.63 log cfu/ml, 3.60 log cfu/ml and 4.46 log cfu/ml for U1, U2 and U3 respectively and differed significantly ($p < 0.05$). Sample U3 had a significantly ($p <$ 4.46) higher yeast count than U1 and U2 respectively.

Multiplication of yeasts is fuelled by endogenous amylolysis, which converts cooked starch to fermentable sugars. The fermentation period has a large impact on the quality of the product. Due to the presence of LAB, the lower the alcohol concentration, pH, and viscosity, the longer the fermentation was permitted to continue (Hlangwani, et al., 2021:1). The observed yeast counts reported in this study are lower in comparison to the 6.42-7.10 log cfu/ml, reported by Katangole, (2008:66) . Which may be due to the fermentation time. In this study, the fermentation period was 24 hours and sorghum, maize meal and maize malt was used as ingreadents, as compared to the 48 hours and only sorghum malt and maize meal was used as ingredients by Katangole, (2008:66). According to Adekoya et al. (2018:23), *S. cerevisiae* is one of the most dominant organisms in the spontaneous fermentation of Umqombothi. Yeast counts are higher than most other microorganisms, except for LAB and TVCThe strains of this specie influence the flavour and aroma characteristic of Umqombothi (Walker & Stewart, 2016). Yeast thrives at a high water activity, warm acidic environment (20-30^{o}C; pH 4.5-6.5), while moulds grow between pH 2-8.5 (Livens, 2015:339; Walker & Stewart, 2016:2). Yeast is the only living microorganism that can change from respiratory to fermentation metabolism. If glucose is present, it will always take a fermentative route to utilize it, despite the availability of oxygen (Russell, 2016:81). *Saccharomyces cerevisiae* traditionally

conducts top fermentations where yeasts congregate on the surface of the fermenting wort (Livens, 2015:341)-. These growth conditions of yeast and moulds render Umqombothi a selective environment for their growth. Umqombothi exhibited yeast and mould counts of 3.41 log cfu/ml, 3.52 log cfu/ml, 5.75 log cfu/ml and 2.14 log cfu/ml, 2.61 log cfu/ml, 0.00 log cful/ml for yeast and mould at U1, U2 and U3 fermentation temperatures, respectively. The microbial counts in the final product result in Umqombothi being susceptible to rapid deterioration, and a possible explanation for the short shelf life of 2-3 days (Blandino et al., 2003:529; Bentley, 2006:35).

Traditional fermented beverages could be a valuable supply of yeast for the brewing industry. The role of a yeast strain in product quality is frequently underestimated, yet it has a significant impact on beverage character as a raw material (Cubillos et al., 2019:386). Most microorganisms in Umqombothi are derived from raw materials, such as sieving (removal of solid particle, resulted in a in reduction of the microorganism counts . The impact of particle size was observed in this study. Show that the lower the pH the lower the yeast counts withU1 yeast counts 3.41 log cfu/ml, 3.52 log cfu/ml and 5.75 log cfu/ml, pH 3.96, 4.12 and 4.34 for U1, U2 and U3. The mould counts during Umqombothi production for different fermentation temperatures are displayed in Table 3.12**.** The mould counts of sampling stages BFF, AFF, BSF, ASF and FP ranged from 0.00-6.81 log cfu/ml, 0.00-6.73 log cfu/ml and 0.00-6.77 log cfu/ml for U1, U2 and U3, respectively. The mould counts of the respective sampling stages differed significantly ($p < 0.05$) for U1 and U2. There was no significant difference in mould count between BSF (0.00 log cfu/ml)-FP (0.00 log cfu/ml) sampling stages for U2. The mould count significantly ($p < 0.05$) increased after the fermentation stages for U1, U2 and U3 respectively. The final product sampling stage mould count significantly ($p < 0.05$) decreased in U1 while non was detected in U3. Mould counts for U2 final product sampling stage significantly ($p < 2.62$) increased. The significant ($p < 0.00$) reduction in the microbial count to a point that it is non-detectable at BSF for U1, U2 and U3, could be due to cooking time and temperature. The purpose of cooking is to reduce the number of mould (Russel, 2003:11). The decrease in nutrients, due to microorganism competition of food and lower pH of the final product and the removal of solid particles affected mould counts and significant ($p < 0.05$) increase for U2.

The overall fermentation temperatures values for mould count were 2.98 log cfu/ml, 2.79 log cfu/ml and 2.69 log cfu/ml for U1, U2 and U3, respectively and they were significantly ($p < 0.05$) different. The U1 exhibited a significantly ($p < 2.98$) higher mould count than U2 and U3, respectively. Umqombothi is susceptible to rapid deterioration due to mould growth, which could be one of the reasons Umqombothi has a shelf life of 2-3 days (Blandino et al., 2003:529; Bentley, 2006:35).

Table 3.12: The effect of two-stage fermentation on mould values of Umqombothi

	Mould (cfu/ml)		
Sampling stage	**U1 (30-30°C)**	**U2 (30-25°C)**	**U3 (25-30°C)**
Before first fermentation (BFF)	3.04 ± 0.04[a]	2.91 ± 0.02[a]	3.09 ± 0.08[a]
After first fermentation (AFF)	6.81 ± 0.13[b]	6.73 ± 0.15[b]	6.77 ± 0.28[b]
Before second fermentation (BSF)	0.00 ± 0.00[c]	0.00 ± 0.00[c]	0.00 ± 0.00[c]
After second fermentation (ASF)	2.88 ± 0.03[d]	1.73 ± 0.05[d]	3.60 ± 0.52[d]
Final product (FP)	2.14 ± 0.12[e]	2.62 ± 0.15[e]	0.00 ± 0.00[c]
Overall fermentation temperatures	2.98 ± 2.28[a]	2.79 ± 2.29[b]	2.69 ± 2.63[b]

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

The total coliform count during Umqombothi production for different fermentation temperatures are displayed in Table 3.13. Coliforms that occur in large numbers guide the presence of potential entero-pathogens (*E. coli*) in drinking water, soil and vegetables, indicative of their microbiological quality (Papazian, 2006: 93; Hill, 2016:294). Coliforms can be described as aerobic and facultative anaerobic bacteria, and for these organisms to multiply, they need to ferment lactose under gassy and acidic conditions in a temperature range of 35-37°C (Eaton, 2006:284; Hill, 2016:297). The total coliforms of sampling stages of BFF, AFF, BSF, ASF and FP rage from 0.00-4.41 log cfu/ml, 0.00-4.26 log cfu/ml, and 0.00-7.73 log cfu/ml for U1, U2 and U3 respectively. The total coliforms of BFF and AFF sampling stages were significantly ($p < 0.05$) different for U1, U2 and U3 respectively. The total coliforms significantly ($p < 0.05$) increased after the first fermentation sampling stages for U1, U2 and U3.

Consequently, the evaluation of total coliforms in Umqombothi was necessary, as water, sorghum malt were one of the main ingredients in the production of Umqombothi (Eumann & Schaeberle, 2016:98). Total coliforms were significantly ($p < 0.00$) non-detected at BSF, ASF and FP sampling stages for U1, U2 and U3 respectively. These finding demonstrated that certain stages of heating and fermenting have a critical role in the reduction of coliform bacteria during the production process (Bayoï & Etoa, 2021:12).

Table 3.13: The effect of two-stage fermentation on total coliforms values of Umqombothi

	Total coliforms (cfu/ml)		
Sampling stage	**U1 (30-30^{o}C)**	**U2 (30-25^{o}C)**	**U3 (25-30^{o}C)**
Before first fermentation (BFF)	3.39 ± 0.14^{a}	4.26 ± 0.05^{a}	3.78 ± 0.04^{a}
After first fermentation (AFF)	4.41 ± 0.06^{b}	3.80 ± 0.57^{b}	7.73 ± 0.08b
Before second fermentation (BSF)	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00c
After second fermentation (ASF)	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Final product (FP)	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Overall fermentation tempeartures	1.51 ± 1.93^{a}	1.61 ± 2.06^{a}	2.30 ± 3.19^{b}

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

The overall fermentation temperatures values of total coliforms were 1.51 log cfu/ml, 1.61 log cfu/ml and 2.30 log cfu/ml for U1, U2 and U3, and they were significantly ($p < 0.05$) different. U3 exhibited a significant ($p < 2.30$) higher total coliform count than U2 and U1, respectively. The higher fermentation temperature in Umqombothi production process did not favour the coliform growth. This condition is the reason for the significantly ($p < 0.05$) decrease in coliforms at U1.

3.8.4 Sensory characteristics of Umqombothi

The results of the sensory evaluation of Umqombothi as affected by two-stage fermentation process in three different fermentation temperatures are presented in Figure 3.3 and Table 3.14. The Umqombothi appearance distributions (Figure A) (30-30^{o}C), (30-25^{o}C), and (25-30^{o}C) are fairly symmetric, and the median of consumer panelists is the same, 4-Like moderately. The interquartile range for (30-30oC) and (30-25^{o}C) is approximately 2, while the interquartile range for (25-30^{o}C) is approximately 1.5. The range for (30-30^{o}C) is 3, while the range for (30-25^{o}C) and (25-30^{o}C) is 4. In terms of appearance, all three products had the same median (average). According to the appearance data, (30-25^{o}C) and (25-30^{o}C) are more spread, with a larger spread of the middle 50% for (30-30^{o}C) and (30-25^{o}C). Overall, the appearance ranged from (30-25^{o}C).

The median number of consumer panelists for colour (Figure B) is the same for all three Umqombothi samples, 4-Like moderately. The interquartile for all three Umqombothi samples was one, and the ranges for (30-30^{o}C) and (25-30^{o}C) were two, while the range for (30-25^{o}C) was three. According to the colour data, the spread of the middle 50% was the same for all three samples, with (30-25^{o}C) being the most spread. The (30-30^{o}C) sample had a higher

median, resulting in a better aroma average (Figure C) of 5-Like very much than the (30-25ºC) and (25-30ºC) samples of 4-Like moderately. Based on the total data, the sample aromas are evenly distributed, with the middle 50% having a larger spread (30-30ºC).The (30-30ºC) sample has a higher median, resulting in a better taste average (Figure D) of 4-like moderately than the (25-30ºC) and (30-25ºC) samples, which have 3-Neither like nor dislike. According to the data, (30-25ºC) taste is more widely distributed than (30-30ºC) and (25-30ºC), respectively. The middle 50% spread is wider in (30-30ºC). For the most part, the values are on the upper end of this range (25-30ºC). The upper and lower outliers observed in (25-30ºC).

Samples (30-30ºC) and (25-30ºC) have a texture median (average) of (Figure E) 4-Like moderately compared to (30-25ºC) of 3-Neither like nor dislike. According to the data, (25-30ºC) has the largest range, thus more spread, and the spread of the middle 50% is larger. The samples (30-30ºC) and (25-30ºC) have a higher overall acceptability median (average) of (Figure F) 4-Like moderately than the sample (30-25ºC) of 3-Neither like nor dislike. According to the data (30-25ºC), overall acceptability has a wider range and thus is more spread. Because the sample (30-25ºC) has an interquartely of 1.5, the spread of the middle 50% is greater.

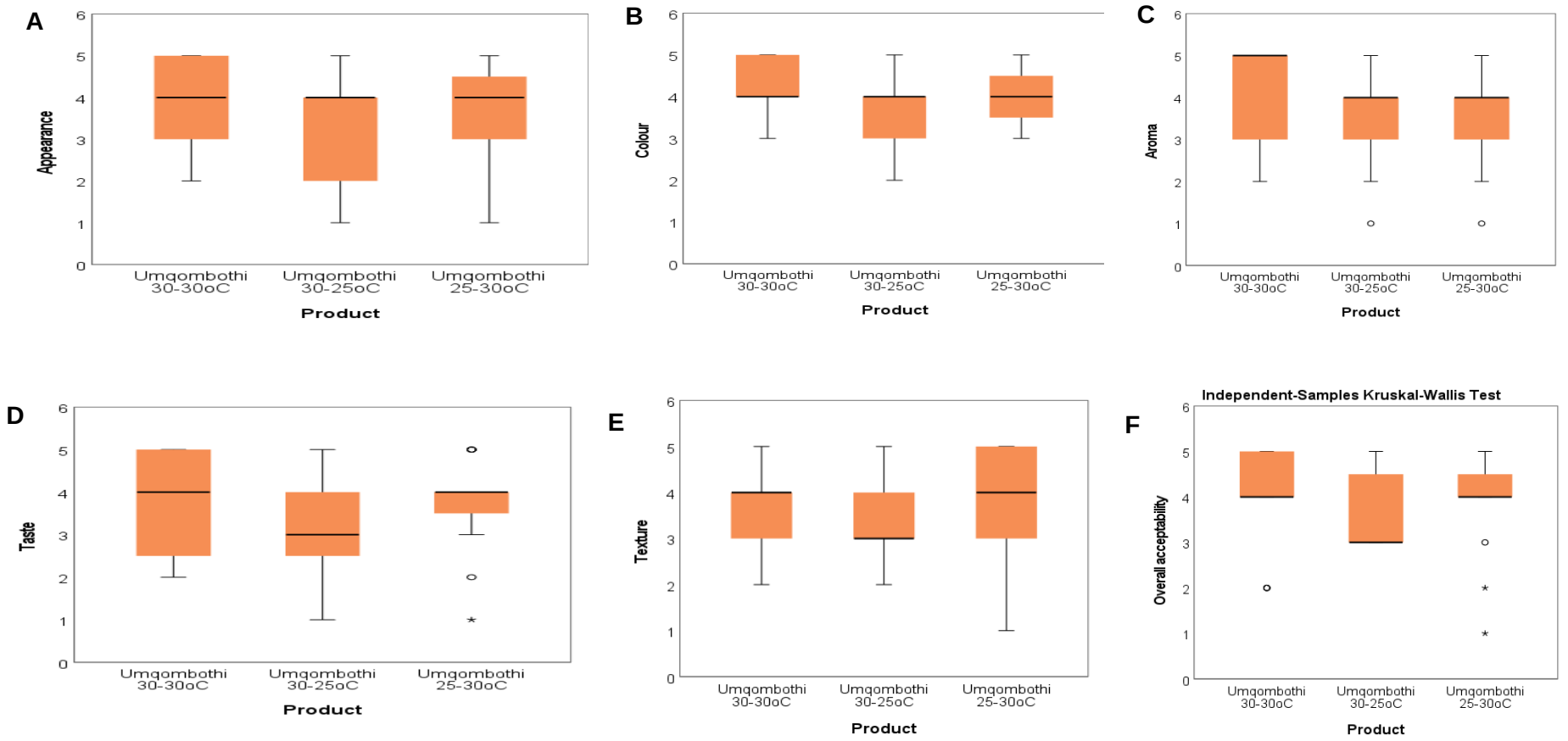

Figure 3.3: Kruskal willis test for sensory attributes, scale of 1 to 5 (1- Dislike very much, 2-Dislike moderately, 3-Neither like or Dislike, 4-Like moderately and 5-Like very much) (n=50)

Table 3.14: Umqombothi Kruskal ranks

	Umqombothi		
Sensory attributes	**U1 (30-30°C)**	**U2 (30-25°C)**	**U3 (25-30°C)**
Appearance	25.43[a]	19.10[a]	24.47[a]
Colour	28.20[a]	19.00[a]	21.80[a]
Aroma	26.90[a]	21.50[a]	20.60[a]
Taste	25.77[a]	18.63[a]	24.60[a]
Texture	24.10[a]	18.70[a]	26.20[a]
Overall acceptability	25.93[a]	19.97[a]	23.10[a]

Mean value ± standard deviation of triplicate determination. Mean values in the same row followed by different letter are significantly ($p \leq 0.05$) different. Sensory distribution for attributes

3.9 Conclusion

Umqombothi was succefully produced from three different fermentation temperatures, using a two-stage fermentation process. Sample U1(30-30°C), appears to be the correct approach to ferment Umqombothi with the lowest pH which supresses the growth of spoilage microorganism and exhibitspositive effect on the shelf life of the fermented beverage. More total soluble solids were detected on U1, which acts as a nutrient source for LAB and yeast to produce flavour aroma, taste, texture and alcohol during the production process. Sample U1(30-30°C) fermentation temperatures, influences the sour taste of the Umqombothi beverage and contributes to generating the appearance, aroma, taste, and overall acceptability. According to Briggs et al.*(*2004:590) and Katongole (2008:59) the colour of Umqombothi varies from pale buff to pink-brown to cream-colour after sieving, based on the naked eye. According to the current study, Umqombothi colour results were all positive and lighter in colour, as the lightnes values were more than 70 which is close to 100, which represent whitness in the colour scale, with more yellowness and redness in U1, than other fermentation temperatures. Therefore Umqombothi in this study is light, yellow and red to creamy colour after sieving. However, complementary studies on Umqombothi beverage conservation are needed.

3.10 Reference

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CHAPTER 4: EFFECT OF SORGHUM AND MAIZE MALTS PARTICLE SIZE ON PHYSICOCHEMICAL, STABILITY, MICROBIOLOGICAL AND SENSORY CHARACTERISTICS OF UMQOMBOTHI

Abstract

The consumption of Umqombothi, a traditional indigenous pink cloudy alcoholic beverage produced from sorghum malt in South Africa, is widespread in the township and rural areas. This beverage is consumed in its fermented stage and is popular among South Africans of all ages. The purpose of this study was investigate the impact ofdifferent particle sizes of sorghum and maize malts on the physicochemical, microbiological, stability, and sensory properties of Umqombothi. Three different particles sizes were compared, namely: normal, coarse, and fine powder particle sizes. Subsamples of fermented beer were taken at the following stages during production of Umqombothi: Before first fermentation (BFF), after first fermentation (AFF), before second fermentation (BSF), after second fermentation (ASF) and the final product (FP). Lactic acid bacteria (LAB) were significantly dominant at 8.16, 7.11 and 5.91 log cfu/ml in the Final product, for normal (maize meal, sorghum malt and maize malt, ingredient as they are on the original package), coarse (sorghum malt and maize malt), and fine powder (sorghum malt and maize malt) particle sizes, respectively. The yeast counts were 3.3, 4.64 and 5.63 log cfu/ml for normal (maize meal, sorghum malt and maize malt), coarse (sorghum malt and maize malt), and fine powder (sorghum malt and maize malt) particle sizes, respectively. Total coliforms and moulds were significantly reduced to non-detectable levels in the Final product, for normal, coarse, and fine powder particle sizes, respectively. The Total soluble solids (TSS) significantly decreased in after second fermentation samples and the coarse particle size exhibited the significantly highest alcohol and significantly lowest pH levels, with no significant difference with fine powder particle size. Umqombothi prepared from the normal particle size to be significantly better than coarse and fine powder particle sizes, respectively. Umqombothi's end product's colour, flavour, and other attributes are influenced by the various particle sizes employed in its production, which also has an impact on the product's overall quality. Particle sizes sorghum and maize malts affect the Umqombothi production process and the quality of the final product, as well as yeast and LAB growing symbiotically during Umqombothi production process.

4.1 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 (Bayoï & Etoa, 2021:74). Traditional beverages from South Africa, for which maize or sorghum are valuable raw material, are known as Umqombothi (Bvochora & Zvauya, 2001:365). Starch are significant component in Umqombothi production, as it not only serves as a source of

carbohydrate but also aids as a thickening and suspending agent. These particles are kept in suspension through the gelatinization of starch, consequently giving the beverage a characteristic creamy appearance. However, complete conversion of starch is avoided (Briggs et al., 2004:590; Lyumugabe et al., 2012:513; Konfo et al., 2015:192). Also, the Umqombothi fermentation process is traditionally performed under uncontrolled conditions, adding to the challenge to produce a beverage with an extended shelf life (Lyumugabe et al., 2012:590; Konfo et al., 2015:190; Adekoya et al., 2018:21). Production of Umqombothi involves typically lactic acid and alcoholic fermentation. Due to their higher growth rate, the lactic acid bacteria (LAB) fermentation dominates the early stages, while yeasts gradually build their way up to the latter part of the complete fermentation process (Usai et al., 2013; Simatende et al., 2015; Cason et al., 2020; Hlangwani et al., 2020). While it largely remains a traditional process, the brewing of Umqombothi attained significant enhancements in scientific knowledge and technology over the last century. There are wide range of technical, biochemical, microbiological and genetic inventions in the modern malting and brewing industry (Mousia et al., 2004: 2213). Transgenic malts and appropriate starter cultures in malting offer intriguing new possibilities for ensuring balanced enzyme activity and avoiding harmful fusarium contamination (Linko et al., 1998: 88). Several genetically modified brewer's yeasts, such as yeasts encoding -acetolactate decarboxylase and super-flocculating yeasts, have been developed (Linko et al., 1998). These and other advancements generally fail to adequately address the effect of malt milling to particle sizes on the quality of Umqombothi. In contrast to other traditional beers in Africa, sorghum and maize malt are not milled or graded during the Umqombothi production process (Mousia et al., 2004:2213; Niemi, Craig B Faulds, et al., 2012:155). There is no research on the effects of varying particle sizes in Umqombothi Milling reduces particle sizes to the micron level, which enhances carbohydrate solubility yield through the release of several enzymes, milling causing physical breakdown, which liberates soluble carbohydrate without the need for external enzymatic treatment. Starch hydrolysis is greatly influenced by its physical state. Degradation of starch increases water absorption and enzymatic sensitivity, influencing the physicochemical qualities of starch as well (Mousia et al., 2004:2214; Niemi, Craig B Faulds, et al., 2012:155).

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 (Mousia et al., 2004:2214). 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 (Mousia et al., 2004:2214; Langenaeken et al., 2019:138). The most commonly used enzymes in brewing for the hydrolysis of wort are α-amylase, β-amylase, and endo-β-1,3:1,4glucanases (Mousia et al., 2004:2213; Bentley, 2006:35). Because sorghum beverages have played such a significant role in traditional

civilization, it is critical to understand the impact of particle size of malts. However, the number of studies conducted in this area is limited, particularly information on the effect of particle sizes in 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.

4.2 Material and Methods

4.2.1 Source of material and equipment

Maize meal, sorghum malt, maize malt, and Mnanti beer powder were obtained from a local supermarket in Bellville, South Africa. Maize malt (Umthombo wombona) was purchased from Boxer Supermarket, Eastern Cape Province (Mdatsane), South Africa. Plate Count Agar ("PCA", NCM0010A, Neogen culture media, Lasec South Africa), De Man Rogosa and Sharpe ("MRS", NCM0190A, Neogen culture media, Lasec South Africa), Violet Red Bile Agar ("VRBA", biolab, Merck South Africa) and Rose-Bengal Chloramphenicol Agar ("RBC", NCM0135A, Neogen culture media, Lasec) were obtained from Merck (Cape Town, South Africa). Sensory evaluation was conducted on Umqombothi produced in the laboratory with 30-30°C fermentation temperatures and coarse particle size. Umqombothi was purchased in the township of Mbekweni (Langabuya) and New-Rest (Ezimbacwini) in Cape Town, Paarl under Drakenstein Municipality. All brewing equipment used in Umqombothi production was acquired from the Department of Food Science and Technology, Cape Peninsula University of Technology (CPUT), South Africa.

4.2.2 Milling of sorghum and maize malt

Sorghum and maize malts were milled using a two-roll mill (Stake STR100A U with fluted rolls) to obtain two particle sizes (coarse and fine powder). The configuration and setting of roller miller were establish according to pre-trails (Rumler et al., 2021: 13), which is the lab scale roller system (results not presented here). The rollers gap of 1 mm for coarse size and 0.5 mm for fine powder for both sorghum and maize malt particle sizes were set using an Allen-key. Coarse and fine powder particle-sized sorghum and maize malts were obtained. Samples of and stored at room temperature. Different particle size of sorghum malt and maize malt after milling (Figure 4.1)

Figure 4.1: Normal, Coarse and fine particle-sized sorghum and maize malts were obtained

4.3 The Umqombothi production process

Figure 4.2 clearly illustrated the the combine different ingredients of the same particle sizes. The Umqombothi production flow diagram employed in this chapter is presented in Figure 4.3. Three batches of Umqombothi were produced by weighing out 500 g of maize meal, 100 g of sorghum malt (normal" are the ingredient as they are on the original package", coarse, and fine powder) and 100 g of maize malt (normal, coarse, and fine powder) in three separate containers labelled normal, coarse, and fine powder particle size. Thereafter, 5 l of lukewarm warm water was added to each container and mixed to form a homogenous mixture. The three batches were incubated for 24 hours at 30^{o}C for the first fermentation to occur naturally. After 24 hours, the three batches separated into two phases, namely the supernatant and the sediment. The supernatant (liquid) of the respective batches was decanted into separate stainless-steel pots and heated to boiling point. The sediments (solid particles) from each batch were added to the boiling liquid. The temperature was then reduced to 60^{o}C while stirring for 40 minutes until a porridge was formed. The porridge was cooled to room temperature, and 300 g of sorghum malt (normal, coarse, and fine powder) and 75 g of Mnanti beer powder in 250 ml water used as inoculum and the mixture incubated at 25^{o}C for 24 hours before being added to the room temperature porridge. The three batches were incubated for 24 hours at 30^{o}C for the second fermentation. Thereafter, the products were removed from the incubation chamber and strained through a sieve with a pore size of ca 0.55 mm. The supernatant of each

batch, referred to as Umqombothi, was transferred to a labelled sterile container while the sediment was discarded (Katongole, 2008:7).

4.3.1 Sampling

Triplicate samples for each batch were collected before first fermentation (BFF) and after first fermentation (AFF), before second fermentation (BSF), after second fermentation (ASF) and final product (FP). The samples were collected using a sterile sampling cup and stored at refrigeration temperature (4-6^{o}C) before analysis. The samples were evaluated for chemical, physicochemical, microbiological and sensory analysis. A sensory evaluation was conducted on final product samples for each batch.

Figure 4.2: Combination of the dry ingredients (maize meal, sorghum and maize malt) for A (normal), B (coarse), and C (fine powder particle sizes) for production of Umqombothi

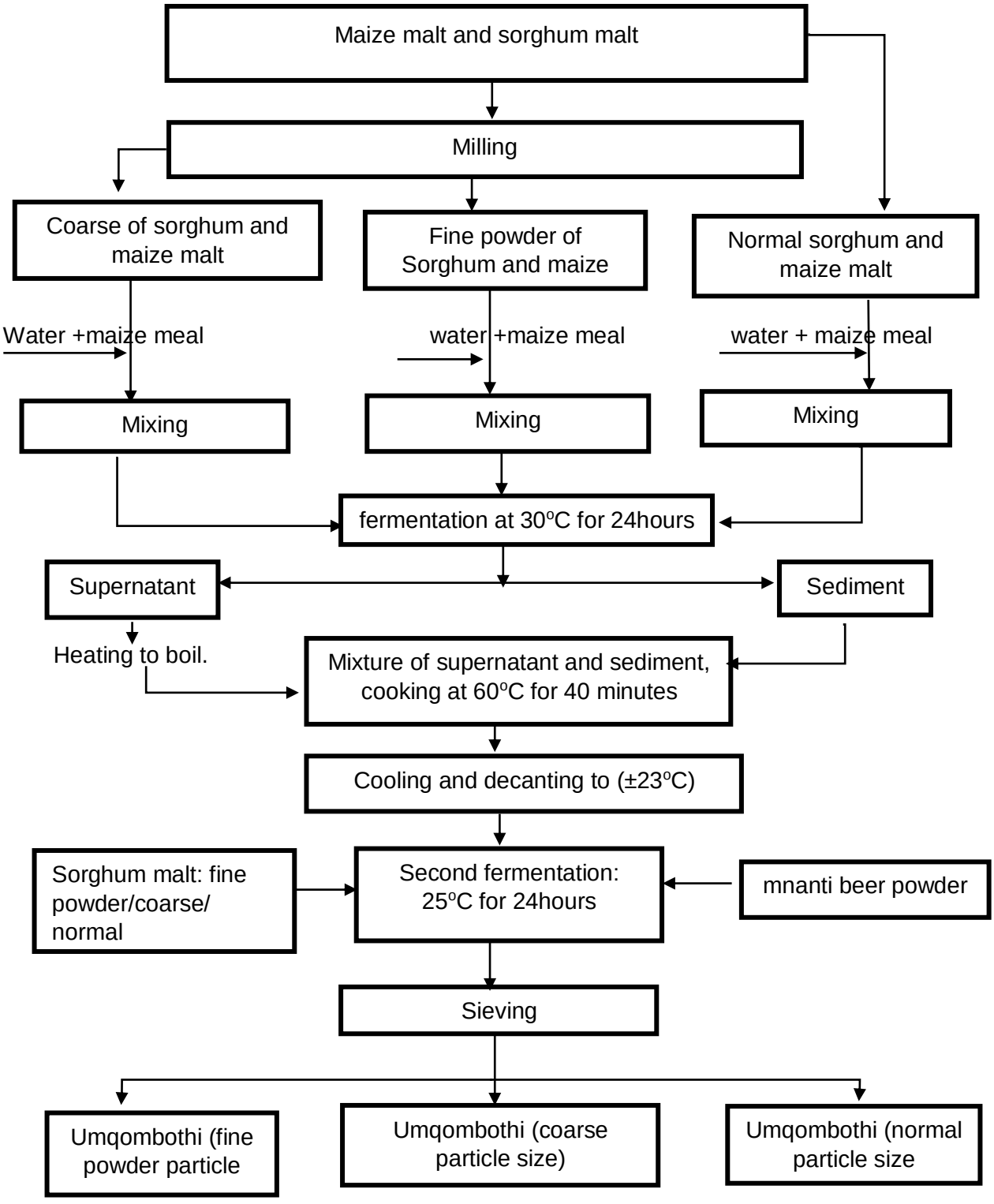

Figure 4.3: Flow diagram to produce Umqombothi from different particle sizes (normal, coarse, and fine powder) of sorghum and maize malt

4.4 Chemical analysis

4.4.1 pH analysis of Umqombothi

The pH for each sample was measured at ambient temperature (23 ± 2°C) using a calibrated pH meter from Mettler-Toledo GmbH, Switzerland (FiveEasy F20) (Attchelouwa et al., 2017:2)

4.4.2 Determination of Total soluble solids (TSS) and Specific gravity (SG) in Umqombothi

TSS was measured with a refractometer (Bellingham & Stanley, serial no 036906, UK, 0 -50% °Brix). The gravity was measured using a Brew craft refractometer [Portable °Brix & Beer wort specific gravity refractometer, handheld (°Brix 0-32, and Gravity 1.000-1.130)]. Several drops of the sample were placed on the prism surface. The liquid on the prism must be free of bubbles or floating particles of pulp or other matter. The prism wasthen closed. To get a valid reading, the instrument wasurned towards the light. If necessary, the eyepiece wasfocused until a clear image appears. The position at which the demarcation line and dark regions cross the vertical scale, it the value of the percentage of total soluble solids reading (Attchelouwa et al., 2017:2).

4.4.3 Determination of alcohol content during Umqombothi production process

Ten millilitres of each sample were centrifuged at 11000 rpm for 10 minutes in an Avanti J-E Centrifuge (Beckman culture, USA),. The supernatants acquired from each sample were used for alcohol content determination by gas chromatography (GC) according to the methods of (Katongole, 2008:62). (Gas Chromatograph system 7890A, Agilent Technologies,. Ethanol 96% was diluted into 2%, 4%, 6%, 8% and 10% for calibration, with 0.99 corelation.

4.5 Physical characteristics of Umqombothi

4.5.1 Colour measurements of Umqombothi

The colour of Umqombothi was evaluated using a Konica Minolta Spectrometer CM 5 [Norich (pty) LtD (Japan)], 45°/0° standards, set at standard observer 10° and D65. The instrument was zero calibrated using 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). Umqombothi (3 g) was deposited in a light-coloured sample holder, and the reflection was measured on the L* a* b* and LCh colour scales. The L* coordinate is lightness, where a value of 100 represents whiteness, and 0 blackness. Coordinate a* referred to green (-)/red (+) chromatic and coordinate b* referred to blue (-)/yellow (+) chromatic. Measurements for each sample was performed in triplicate, C* (Chroma, and h (hue) angle 0°=+a*, 90=+b*, 180=-a* and 270°=-b*). The total colour difference of Umqombothi (ΔE*) was calculated by applying the following equation 4.1(Hardy & Jideani, 2019:3).

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

4.5.2 Measuring syneresis (STS) of Umqombothi

The method of Samson A Oyeyinka et al., (2016:122) was used to determine syneresis in the Umqombothi samples after storage at 4°C for 2–3 days. The samples were centrifuged at 350 g for 10 min at 4°C in an Avanti J-E Centrifuge (Beckman culture, USA). Triplicate analysis was performed and the following equation was used to calculate Syneresis (STS).

$$\%STS = \frac{V1}{V2} x\ 100 \quad \text{Equation 2}$$

V_1 = Volume of Umqombothi whey collected after drainage

V_2 = Volume of Umqombothi beer

4.5.3 Determination of the viscosity of Umqombothi traditional beer

The change in viscosity of Umqombothi over time was determined at before first fermentation (BFF) and after second fermentation (ASF) sampling stages, using a Rheolab QC (Anton Paar, 81602957) with temperature device C-PTD 180/AIR/QC, 81622948 and measuring system CC27, (Austria). The beverage (18 ml) was poured into an upward projected sample cup and analysed following the manufacturer's instruction at 25-4°C for 17 min. In all runs, the shear rate (s^{-1}) was kept constant . Vescosity analysis was performed in triplicate (Jideani *et al.*, 2021:6).

4.6 Microbiological analysis

4.6.1 Enumeration of microorganisms in Umqombothi

Enumeration of the total viable count (TVC), LAB, Coliforms, and yeast and moulds in Umqombothi were performed according to the methods described by SANAS 4833:2007, ISO: 4833:2007 The pour plate method was employed for the enumeration of microorganisms in Umqombothi. Counting of all typical colonies was performed using a ColonyStar "colony counter "from Funke GERBER labortechnik (Berlin, Germany), and control (Positive controls for VRBA, MRS, RBC, and SPCA performed by streaking with *Escherichi. coli*, Lactobacillus. Gasseri, and yeast (*Saccharomyces cerevisiae*), mould (*aspergillus*). Controls for (ringer, stomacher bags and pippette tips) were carried out with 1 ml of ringer into a Petri dish plate (ringers' control), stomacher bag (control), and pipette tips (control) and poured with PCA and incubated at 37°C for 24 hours. Experiments were performed in triplicates. Only plates containing colonies from 30-300 were counted (Hardy & Jideani, 2019:5).

4.6.2 Bacterial enumeration

Umqombothi (10 g) was weighed into 90 ml sterile ringers' solution (10^{-1}) and mixed well. Then a series of dilutions (10^{-1} to 10^{-6}) were prepared. For each dilution, 1 ml aliquot was carefully and aseptically transferred into the base of four labelled sterile Petri-dishes. For each dilution,

approximately 12-15 ml of pre-cooled plate count agar (PCA), de Man Rogosa and Sharpe Agar (MRS) and violet-red bile agar (VRBA) were poured into each petri dish, respectively. After that, the plates were carefully swirled to mix well. Once all plates were allowed to solidify, they were incubated in an inverted position. The MRS, PCA and VRBA plates for each dilution were incubated at 37°C for 48 hours (katongole, 2008:62).

4.6.3 Enumeration of yeast and moulds

From the series of dilutions in 4.6.2, 1ml aliquote was thereafter aseptically transferred to pre-labelled sterile petri dishes and approximately 12–15 ml of pre-cooled Rose Bengal chloramphenicol agar (RBC) was poured into each petri dish plate and swirled to mix well. Once solidified, plates were incubated at 25°C for 5 days in an inverted position (Katongole, 2008:62).

4.7 Sensory evaluation

Sensory evaluation was conducted based on the IFST Guidelines for Ethical and Professional Practices for the Sensory Analysis of Foods. To reduce risks to participants, Umqombothi was assessed for potential microbial, chemical, and physical hazards and certified safe before conducting the sensory evaluation. Ingredients and manufacturing process are similar to what commercial Umqombothi is made. Research activities were restricted to those detailed in the research proposal. Test participants were volunteers, and their right to withdraw from the research was explained to them; informed consent was obtained from research participants, the product ingredient and alcohol content was explained to participants and their anonymity and confidentiality was protected. Umqombothi is a traditional South African alcoholic beverage with an alcohol concentration of 2-3 %. Because of the dangers of drinking to one's health and that it impairs one's ability to drive. Panellists were limited to one round of tasting, which consisted of 90 ml of Umqombothi or around 1.8 ml of alcohol per individual. Panellists were also be told that they are not required to finish the product because the goal was to taste rather than drink.

A consumer sensory analysis was performed on the three batches of Umqombothi using 50 untrained panellists in the Sensory Laboratory of the Food Science and Technology Department of the Cape Peninsula University of Technology. The samples were presented in 50 ml white polystyrene cups placed side by side on a plastic tray. Each sample cup contained 30 ml of the respective Umqombothi batches and was identified by a three-digit code and served at room temperature (23 ± 2°C). A cup of water was provided to clear the pallet before and during tastings. The panellists were provided with a score sheet that consisted of three coded samples with a 5-point hedonic scale ranging from 1= dislike extremely to 5 = like extremely, according to Salmerón et al. (2015:109). The panellists were instructed to rate each sample on its merit on the five-point hedonic rating scale for appearance, colour, taste, aroma,

texture, and overall acceptability. The preferred particle size batch based on the sensory evaluation was used during the subsequent production of Umqombothi using (Attchelouwa et al., 2017:3).

4.8 Data analysis

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

4.9 Results and Discussion

4.9.1 Effect of particle size on the physicochemical characteristics of Umqombothi

During the production of Umqombothi, there were five different sampling stages. Before the first fermentation (BFF), after the first fermentation (AFF), before the second fermentation (BSF), after the second fermentation (ASF), and finally the final product (FP).

The pH obtained during the manufacturing process of Umqombothi is presented in Table 4.1. The pH of sampling stages BFF, AFF, BSF, ASF and FP ranged from 3.45-6.03, 3.41-6.02 and 3.46-5.96 for normal, coarse, and fine powder particle sizes, respectively. There was a significant ($p < 0.05$) difference in the pH between all sampling stages for normal particle sizes. There was no significant difference between ASF-FP sampling stages of coarse and fine powder particle size. The pH decreased significantly ($p < 0.05$) between BFF and FP sampling stages for normal particle sizes. There was a significant ($p < 0.05$) decrease between BFF-ASF sampling stages for coarse and fine powder particle sizes. The significant ($p < 0.05$) decrease in pH is due to significant increase in the number of LAB during the Umqombothi production process, specifically at the BFF stages (5.02, 4.99, and 5.09 log cfu/ml) and at the FP (8.16, 7.11, and 5.91 log cfu/ml) for normal, coarse, and fine powder particle sizes. Lactic acid bacteria produced lactic acid and acetic acid, causing the pH to significantly decrease. The pH of the FP sampling stages, i.e., 3.45, 3.46, and 3.49 for normal, coarse, and fine powder particle sizes, respectively, are in accordance of results reported for Umqombothi ((Adekoya et al., 2018: 25; Katongole, 2008: 65; Hlangwani et al., 2020: 6). The current study confirmed that particle size influenced the pH of Umqombothi during the production process. The normal particle size (not milled maize malt and sorghum malt) resulted in the preferred low pH (3.45) Umqombothi than coarse (3.46) and fine powder (3.49). The fine powder particle sizes, FP sampling stage pH significantly ($p < 0.05$) higher as compared to normal and coarse particle sizes with a lower pH level. The lower the particle size, the higher the pH level for FP sampling stage. Beverage with significant low pH has a longer shelf life, superior safety, and quality, as well as antimicrobial properties.

Table 4.1: The effect of particle sizes on pH value of Umqombothi

Sampling stage	pH		
	Normal	Coarse	Fine powder
Before first fermentation (BFF)	6.03 ± 0.01[a]	6.02 ± 0.01[a]	5.96 ± 0.01[a]
After first fermentation (AFF)	3.55 ± 0.02[b]	3.41 ± 0.01[b]	3.46 ± 0.01[b]
Before second fermentation (BSF)	4.07± 0.02[c]	4.02 ± 0.01[c]	3.97 ± 0.01[c]
After second fermentation (ASF)	3.54 ± 0.01[d]	3.47 ± 0.01[d]	3.48 ± 0.01[d]
Final product (FP)	3.45 ± 0.00[e]	3.46 ± 0.01[d]	3.49 ± 0.00[d]
Overall particle sizes	4.13 ± 1.01[a]	4.08 ± 1.03[b]	4.07 ± 0.99[b]

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

Those beverages assist in the removal of harmful microorganisms that may constitute a health risk (Rogers et al., 2016:152). When comparing the impact of pH as affected by fermentation temperature, there was no significant difference between the overall particle sizes pH of coarse (4.08), and fine powder (4.07). The normal particle size was significantly ($p < 4.13$) higher than coarse and fine powder particle sizes. Beverages with a lower pH (4.08), such as coarse particle size, have a longer shelf life, superior safety and quality, and higher antibacterial properties according to Hlangwani et al., (2020:3). Those beverages qualities aid in the removal of harmful microorganisms that may constitute a health risk (Hlangwani, et al., 2021:1). The overall particle sizes pH for normal, coarse, and fine powder particle sizes, respectively, are higher than the pH of 3.67 reported by Adekoya et al. (2018:25) and Katongole, (2008:65) on the final product of Umqombothi. This could be due to the fermentation period, source of the grains and inoculum used

Umqombothi has a moisture content of 94.67% and a limited shelf life of 1-3 days, according to Adekoya et al. (2018:25). Foods with high moisture content are more prone to microbial development, reducing shelf life. The effect of excessive moisture on the shelf life of Burukutu, Ghana's traditional beverage, was studied by Atter et al. (2017:352). Sorghum fermentation usually results in enzyme activation, a decrease in pH, increased metabolic activity, and microbial activity. This causes the breakdown of starch, enhancing nutritional quality (Hlangwani et al., 2020:10). The activity of enzymes is affected by pH, which is essential in liquefaction and the conversion of malt starch into fermentable sugars (Adekoya et al., 2018:25). Furthermore, elevated pH promotes microbial growth by lowering proteinase and amylase activity and stability (Hlangwani et al., 2021:10). According to the current study, the coarse and fine powder Umqombothi batch produced a significantly ($p < 0.05$) lower pH compared to normal particle sizes Umqombothi batch.

The total soluble solids as affected by different particle sizes during Umqombothi production are presented in Table 4.2. The total soluble solids (TSS) of sampling stages BFF, AFF, BSF, ASF and FP ranged from 0.00-9.83, 0.00- 9.93, and 0.00-10.00°Brix for normal, coarse, and fine powder particle sizes, respectively. There was a significant ($p < 0.05$) difference between all sampling stages for normal, coarse, and fine powder particle sizes. The TSS increased ($p < 0.05$) significantly between BFF and BSF sampling stages for all three particle sizes. The endosperm protein that surrounds the starch granule softens during gelatinization, which results in a transfer of the grain to the retting water and subsequent increase in the concertation of total soluble solids. This could explain why TSS increases during fermentation and BSF sampling stages. Cooking the sour porridge long enough allows the starch to gelatinize and the yeast cell's lockup nutrients to be released. The TSS level is influenced by cooking time and particle sizes (Hlangwani, et al., 2021:4). The TSS decreased ($p < 0.05$) significantly between BFF and FP sampling stages for all three particle sizes. Before second fermentation sampling stage, Umqombothi contains a high level of TSS. As the second fermentation progresses, the accessible solids are utilized by microorganism such as LAB and yeast to create alcohol, flavour and aroma (Attchelouwa et al., 2017: 5). A significant ($p < 0.05$) decrease in total soluble solids was observed in the final product of the normal, coarse and fine powder particle size, possibly due to sieving. By removing the second conversion of malt, Kutyauripo et al., (2009:274) observed a similar trend. When samples were held between 28°C and 30°C, Attchelouwa et al., (2017:5) found a decrease in TSS from 9.7 to 8. According to Attchelouwa et al., (2017:5), the greatest reduction in TSS resulted in a more significant bacterial burden.

Table 4.2: The effect of particle sizes on total soluble solids values of Umqombothi

	Total soluble solids (°Brix)		
Sampling stages	**Normal**	**Coarse**	**Fine powder**
Before first fermentation (BFF)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
After first fermentation (AFF)	1.10 ± 0.00^{b}	1.80 ± 0.00^{b}	1.87 ± 0.12^{b}
Before second fermentation (BSF)	9.83 ± 0.06^{c}	9.93 ± 0.12^{c}	10.00 ± 0.00^{c}
After second fermentation (ASF)	4.67 ± 0.29^{d}	5..67 ± 0.29^{d}	6.00 ± 0.00^{d}
Final product (FP)	4.50 ± 0.00^{e}	5.50 ± 0.00^{e}	5.83 ± 0.29^{e}
Overall particle sizes	4.02 ± 3.56^{a}	4.58 ± 3.57^{b}	4.74 ± 3.62^{c}

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

The overall particle size total soluble solids values were 4.02, 4.58, 4.74°Brix and they were significantly ($p < 0.05$) different for normal, coarse, and fine powder, respectively. Umqombothi produced with fine powder particle size had a significantly (4.74) greater TSS than coarse and normal particle sizes. Not all dissolved solids were available for utilization by the microorganisms present, as seen by the levelling of solids in the products at the end of the production process. It is possible that the levelling is due to the inhibiting effects of ethanol. During gelatinization, the amount of TSS increases, transferring the grain to the retting water (Hlangwani, et al., 2021:4). This could explain the rising trend in TSS levels as cooking and fermentation of the fine powder particle size increases. Fine powder appears to be the perfect particles size when it come to TSS.

The specific gravity as affected by different particle sizes during Umqombothi production are presented in Table 4.3. Specific gravity is a measurement that indicates the progress of the fermentation process (Almonacid et al., 2012:271). The more sugar dissolved in the liquid (wort), the greater the SG value. The SG of sampling stages BFF, AFF, BSF, ASF and FP ranged from 1.00-1.04, 1.00-1.11, and 1.00-1.28 for normal, coarse, and fine powder particle sizes, respectively. There was no significant difference between sampling stages of the normal particle size, during Umqombothi production process. There was no significant difference, between BFF-AFF, BSF-ASF sampling stages of coarse, and fine powder particle sizes, during Umqombothi production processes. Final product sampling stage was significantly ($p < 0.05$) higher to all other sampling stage of coarse and fine powder particle sizes. The overall particle sizes specific gravity levels of normal, coarse, and fine powder particle sizes did not differ significantly from each other. Umqombothi produced with fine powder particle size had a higher SG at (FP) than normal and coarse particle sizes. The lower the particle size, the higher the SG at FP sampling stage. The percentage alcohol content by volume (%ABV) of the resulting beverage can be calculated by subtracting the original gravity (OG) from the final gravity (FG) of the wort (Entwisle et al., 2008: 461; Spedding, 2016:126). The increase in SG from unfermented to fermented products followed a similar pattern, according to Atter et al. (2017:351).

The alcohol content as affected by different particle sizes during Umqombothi production are presented in Table 4.4. The % alcohol obtained in sampling stages BFF, AFF, BSF, ASF and FP ranged from 0.00-2.00%, 0.00-3.44% and 0.00-3.28% for normal, coarse, and fine powder particle sizes, respectively. There was no significant difference, between BFF-BSF, and ASF-FP sampling stage for normal particle sizes. The BFF and BSF sampling stages was significantly ($p < 0.00$) lower than all other sampling stages of normal particle sizes, possibly due to evaporation during cooking. There was no significant difference between the BFF-BSF and ASF-FP sampling stages of the coarse, and fine powder particle sizes. The BFF and BSF sampling stages was significantly ($p < 0.00$) lower than all the other sampling stages for the coarse and fine powder particle sizes. The FP sampling stages of coarse (3.44%)

particles sizes exhibited the significantly (p < 3.44) highest alcohol content in comparison with the fine powder (3.28%) and normal (2.00%) particle sizes, respectively. The study confirmed that particle size does affect the alcohol content (Mousia et al., 2004:2216). The normal particle sizes (not milled ingredients, used as they come from the manufacture package) resulted in a low % alcohol, while the coarse and fine powder particle size produced Umqombothi with the higher % alcohol.

Table 4.3: The effect of particle sizes on specific gravity values of Umqombothi

	Specific gravity		
Sampling stages	**Normal**	**Coarse**	**Fine powder**
Before first fermentation (BFF)	1.00 ± 0.00[a]	1.00 ± 0.00[a]	1.00 ± 0.00[a]
After first fermentation (AFF)	1.04 ± 0.05[a]	1.01 ± 0.00[a]	1.01 ± 0.00[a]
Before second fermentation (BSF)	1.04 ± 0.00[a]	1.04 ± 0.00[a]	1.04 ± 0.00[a]
After second fermentation (ASF)	1.02 ± 0.00[a]	1.03 ± 0.00[a]	1.03 ± 0.00[a]
Final product (FP)	1.02 ± 0.00[a]	1.11 ± 0.12[b]	1.28 ± 0.00[b]
Overall particle sizes	1.02 ± 0.02[a]	1.04 ± 0.06[a]	1.07 ± 0.11[a]

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

The % alcohol content contributes to the beverage flavour and impacts its quality (Vanderhaegen et al., 2007:404). The alcohol levels of the final sampling stages for all three particle sizes correspond to those reported by (Hlangwani et al., 2020:6), i.e., between 2-3.5%. The overall particle sizes values alcohol content% of 0.84, 1.44 and 1.39 Umqombothi, as affected by particle sizes of normal, coarse, and fine powder was significant (p < 0.05) different. Coarse particle sizes exhibited the significantly (p < 1.44) highest % alcohol than the fine powder and normal particle size respectively. This can be ascribed to the available solids that are utilized by yeasts and LAB and the subsequent formation of as the fermentation process progresses (Atter et al., 2017:351). The reduction of % alcohol may be related to evaporative ethanol loss at before second fermentation stages, due to cooking (Hlangwani, et al., 2021:2). The % alcohol levels of the overall particle sizes recorded in this study were 1.5 % lower than those reported by (Hlangwani et al., 2020:6), (2-3.5%) and Katongole, (2008:65), (2.6%). The ethanol concentration of Umqombothi will vary depending on where and how it was brewed (Hlangwani et al., 2020:6; Dusabe et al., 2021:2). According to Briggs (2004: 590), the alcohol content ranges from 1-8%, but the most typical range is 2.5-4.5.

Table 4.4: The effect of particle sizes alcohol % of Umqombothi

	Alcohol (%)		
Sampling stages	**Normal**	**Coarse**	**Fine powder**
Before first fermentation (BFF)	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
After first fermentation (AFF)	0.20 ± 0.00[b]	0.33 ± 0.00[b]	0.38 ± 0.00[b]
Before second fermentation (BSF)	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
After second fermentation (ASF)	2.00 ± 0.00[c]	3.44 ± 0.00[c]	3.28 ± 0.00[c]
Final product (FP)	2.00 ± 0.00[c]	3.44 ± 0.00[c]	3.28 ± 0.00[c]
Overall particle sizes	0.84 ± 0.98[a]	1.44 ± 1.69[b]	1.39 ± 1.61[c]

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

According to Entwisle et al. (2008:), microbial conversion of ethanol to acetic acid might induce a decrease in ethanol levels as the beverage ages. Beverage with an alcohol level of 1-2% and 0.5% are commonly classed as low alcohol and non-alcohol beers (Puligundla et al., 2021:526). With a great demand for these brews in the worldwide beverage market. This new trend has evolved due 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 (Hlangwani, et al., 2021:4). With all of that, coarse appears to be the best method for Umqombothi production.

4.9.2 Physical characteristics of Umqombothi

4.9.2.1 Colour characteristics of Umqombothi

The colour attributes of Umqombothi as affected by fermentation and particle sizes were lightness (L*), greenness (-a*), redness (+a*), blueness (-b*), and yellowness (+b*) are presented in Table 4.5.

The lightness of Umqombothi obtained from the different particle sizes, at different sampling stages are presented in Figure 4.10. The lightness of sampling stages BFF, AFF, BSF, ASF and FP ranged from 60.59-92.60, 60.40-93.60 and 49.44-98.76 for normal, coarse, and fine powder particle sizes, respectively. There was no significant difference in lightness between BFF-AFF and ASF-FP sampling stages for the normal particle size. The lightness of all sampling stages of coarse and fine powder particle sizes differed ($p < 0.05$) significantly. Lightness significantly ($p < 0.05$) increased from BSF to ASF for normal, coarse, and fine powder particle sizes. During cooking (BSF) starch is metabolized by yeasts and LAB into

simple sugars and is further converted to alcohol and carbon dioxide, which could result in a significant increase in the lightness of the product (Adekoya et al., 2019:6). Before second fermentation exhibited the lowest lightness levels as compared to all other sampling stages for normal, 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, extended degradation of the starch is avoided during (BSF), as it could cause the beer to be too thin (Konfo et al., 2015:192; Hlangwani et al., 2020:4).

The overall particle sizes values of lightness were significantly ($p < 0.05$) different for normal, coarse, and fine powder. Umqombothi produced with fine powder particle size had a significantly ($p < 0.05$) greater lightness than normal and coarse particle sizes. Fine powder particle sizes resulted in a significant ($p < 0.05$) increase in lightness, as the values were much closer to 100. Milling decreases particle size to the micron level, which increases carbohydrate solubility yield employing multiple enzymes, which liberates soluble carbohydrate without the requirement for enzymatic treatment. Starch degradation improves water absorption and enzymatic sensitivity(Mousia et al., 2004:2214; Niemi, Craig B. Faulds, et al., 2012:155). According to Rumler et al., (2021:6), sorghum lightness (L*value) increased with decreasing particle size, whereas Umqombothi (FP) had the opposite

Table 4.5: The effect of particle sizes on lightness value of Umqombothi

	Lightness		
Sampling stages	**Normal**	**Coarse**	**Fine powder**
Before first fermentation (BFF)	92.60 ± 0.00^{a}	88.90 ± 0.00^{a}	87.18 ± 0.00^{a}
After first fermentation (AFF)	91.80 ± 4.9^{a}	93.60 ± 0.00^{b}	92.13 ± 0.00^{b}
Before second fermentation (BSF)	60.59 ± 0.00^{b}	60.40 ± 0.00^{c}	49.44 ± 0.00^{c}
After second fermentation (ASF)	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 sizes	75.73 ± 14.24^{a}	74.36 ± 14.52^{b}	77.89 ± 19.57^{c}

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

The redness of Umqombothi from different particle sizes, at different sampling stages are presented in Table 4.6. The redness levels of Umqombothi were positive, which suggested that Umqombothi is in the redness colour space. The sorghum kernel was initially reddish, so this was expected (Rumler et al., 2021:6). The redness levels of sampling stages of BFF, AFF, BSF, ASF and FP ranges from 0.64-5.41, 0.22-6.72, and 0.28-9.02 for normal, coarse, and

fine powder particle sizes, respectively. Redness of BFF (0.77) and AFF (0.64) sampling stages of normal particle sizes did not differ significantly. The sampling stages of coarse and fine powder particle sizes were all significantly ($p < 0.05$) different. Milling reduces particle sizes to the micron range, increasing carbohydrate solubility yield using several enzymes. Physical breakdown occurs during milling, resulting in the release of soluble carbohydrates without the need of enzymes (Mousia et al., 2004:2214).

The redness decreased between BFF-AFF normal, coarse, and fine powder particle sizes. This could be due to the fermentation effect on. The same trend was observed between BSF-ASF, which could be ascribed to the addition of sorghum malt before fermentation stages. The concentration of solids in Umqombothi, furthermore, affects the redness of Umqombothi. After removing solids through sieving, the redness levels significantly ($p < 0.05$) increased for normal, coarse, and fine powder particle sizes. The overall redness levels as affected by particle sizes were 3.19, 3.59, 3.42 and they were significantly ($p < 0.05$) different for normal, coarse, and fine powder, respectively. Umqombothi produced with coarse particle size had a significantly ($p < 0.05$) greater redness than fine powder and normal particle sizes, respectively.

Table 4.6: The effect of particle sizes on redness values of Umqombothi

	Redness			
Sampling stages	**Normal**	**Coarse**	**Fine powder**	**Overall sampling stages**
Before first fermentation (BFF)	0.77 ± 0.00^{a}	0.89 ± 0.00^{a}	0.96 ± 0.00^{a}	0.87 ± 0.83^{a}
After first fermentation (AFF)	0.64 ± 0.29^{a}	0.22 ± 0.00^{b}	0.28 ± 0.00^{b}	0.38 ± 0.24^{b}
Before second fermentation (BSF)	5.41 ± 0.00^{b}	5.68 ± 0.00^{c}	9.02 ± 0.00^{c}	6.70 ± 1.74^{c}
After second fermentation (ASF)	4.12 ± 0.09^{c}	4.43 ± 0.48^{d}	0.39 ± 0.00^{d}	2.98 ± 1.96^{d}
Final product (FP)	5.04 ± 0.05^{d}	6.72 ± 0.06^{e}	6.47 ± 0.09^{e}	6.07 ± 0.79^{e}
Overall particle sizes	3.19 ± 2.15^{a}	3.59 ± 2.69^{b}	3.42 ± 3.75^{c}	

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

One of the unique characteristics of Umqombothi is the colour of the brew. The colour of Umqombothi is defined as pale buff, pinkish-brown to cream-colour after sieving (Schaepdrijver, 2004:590; Katongole, 2008:13). Furthermore, sieving fractionation was previously performed for a variety of reasons, including the removal of phytic acid-rich fractions, though others claimed a better nutritional profile when sieving was not used. Sieving

may be useful in improving the quality of certain foods (Rumler et al., 2021:2). This colour definition is based on the observation of the naked eye, as there is no colour statistical research according to our knowledge.

The yellowness of Umqombothi from different particle sizes, at different sampling stages are presented in Table 4.7. The levels were positive (+b*), which suggested that Umqombothi is in the yellowness colour space. This is expected, because yellow maize is mainly used in traditional beer (Katongole, 2008:9; Rumler et al., 2021:6). The yellowness of sampling stages of BFF, AFF, BSF, ASF and FP ranged from 2.44-19.73, 1.54-22.25, and 1.34-24.18 for normal, coarse, and fine powder particle sizes, respectively. Yellowness of BFF-AFF sampling stages of normal particle sizes did not differ significantly. The yellowness levels of all sampling stages for coarse and fine powder particle sizes were significantly ($p < 0.05$) different. Reduction of particle sizes to the micron level, enhances carbohydrate solubility yield using several enzymes. Starch hydrolysis is greatly influenced by its physical state, starch degradation increases water absorption and enzymatic sensitivity (Mousia et al., 2004:2213; Niemi, Craig B Faulds, et al., 2012:155). The yellowness of the before fermentation stages and after fermentation stages exhibited no significant difference for the normal particle size, in contrast to the coarse and fine powder particle sizes, which were significantly ($p < 0.05$) different.

Table 4.7: The effect of particle sizes on yellowness values of Umqombothi

	Yellowness		
Sampling stages	**Normal**	**Coarse**	**Fine powder**
Before first fermentation (BFF)	2.72 ± 0.00^{a}	3.78 ± 0.00^{a}	4.42 ± 0.00^{a}
After first fermentation (AFF)	2.44 ± 1.08^{a}	1.54 ± 0.00^{b}	2.22 ± 0.00^{b}
Before second fermentation (BSF)	19.52 ± 0.00^{b}	20.42 ± 0.00^{c}	24.18 ± 0.00^{c}
After second fermentation (ASF)	19.73 ± 0.12^{c}	19.41 ± 0.64^{d}	1.34 ± 0.00^{d}
Final product (FP)	18.74 ± 0.07^{d}	22.25 ± 0.06^{e}	21.97 ± 0.13^{e}
Overall particle sizes	12.63 ± 8.51^{a}	13.48 ± 9.22^{b}	10.83 ± 10.43^{c}

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

The AFF sampling stages having significantly lower values than BFF for normal, coarse, and fine powder particle sizes. The concertation of solids in Umqombothi do affects the yellowness. Therefore, after sieving, the yellowness significantly ($p < 0.05$) increased for the coarse and fine powder particle sizes and significantly ($p < 0.05$) decreased for the normal particle size. Umqombothi produced with coarse particle size had a significantly greater yellowness at FP

sampling stages than fine powder and normal particle sizes respectively. The yellowness levels for the overall particle sizes were significantly ($p < 0.05$) different for normal, coarse, and fine powder, respectively, with coarse particle sizes having the higher values than normal and fine powder. The colour of Umqombothi is one of its distinguishing features. After sieving, the color of Umqombothi brew is defined as pale buff, pinkish-brown, to cream-colored (Schaepdrijver, 2004:590; Katongole, 2008:13). This colour definition is based on the observation of the naked eye, as there is no colour statistical research according to our knowledge.

The chroma of Umqombothi from different particle sizes, at different sampling stages are presented in Table 4.8. The chroma of Umqombothi was positive during the Umqombothi production process. The chroma of sampling stages of BFF, AFF, BSF, ASF and FP rages from 2.52-20.25, 1.56-23.23, and 1.39-25.80 for normal, coarse, and fine powder particle sizes, respectively. The chroma of ASF-FP sampling stages of normal particle sizes did not differ significantly. The sampling stages of coarse and fine powder particle sizes were all significantly ($p < 0.05$) different. The physical state of starch has a large impact on its hydrolysis; starch degradation increases water absorption and enzymatic sensitivity (Mousia et al., 2004:2213). The chroma decreased ($p < 0.05$) significantly after the fermentation stages for normal, coarse, and fine powder particle sizes. The sieving during the final product sampling stage, resulted in a significant ($p < 0.05$) increase in chroma for coarse and fine powder particle sizes. The concertation of solids in Umqombothi do affect the vividness of Umqombothi, as seen in the effect of sieving. The overall values of chroma as affected by particle sizes were 13.03, 13.96, 11.31 and they were significantly ($p < 0.05$) different for normal, coarse, and fine powder respectively. Umqombothi produced with coarse particle size had a greater chroma than normal and fine powder particles sizes respectively.

Table 4.8: The effect of particles sizes on chroma values of Umqombothi

	Chroma		
Sampling stages	**Normal**	**Coarse**	**Fine powder**
Before first fermentation (BFF)	2.83 ± 0.00^{a}	3.88 ± 0.00^{a}	4.52 ± 0.00^{a}
After first fermentation (AFF)	2.52 ± 1.12^{a}	1.56 ± 0.00^{b}	2.23 ± 0.00^{b}
Before second fermentation (BSF)	20.25 ± 0.00^{b}	21.19 ± 0.00^{c}	25.80 ± 0.00^{c}
After second fermentation (ASF)	20.16 ± 0.14^{b}	19.92 ± 0.50^{d}	1.39 ± 0.00^{d}
Final product (FP)	19.41 ± 0.08^{b}	23.23 ± 0.08^{e}	22.63 ± 0.32^{e}
Overall particle sizes	13.03 ± 8.77^{a}	13.96 ± 9.59^{b}	11.31 ± 11.00^{c}

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

The hue angle of Umqombothi from different particle sizes, at different sampling stages are presented in Table 4.9. The h^o of Umqombothi remained positive during the production process of Umqombothi for normal, coarse, and fine powder particle size. The hue angle sampling stages of BFF, AFF, BSF, ASF and FP ranges from 74.19-78.19, 73.19-81.87, and 69.55-82.90 for normal, coarse, and fine powder particle sizes, respectively. The h^o of BFF-BSF, AFF-FP, BSF-FP sampling stages of normal particle sizes did not differ significantly. The sampling stages of coarse particle size BFF-ASF were not significantly ($p < 0.05$) different. The fine powder particle sizes, sampling stages were significantly ($p < 0.05$) different. More extracts were recovered when the malt was milled to a fine particle size, as expected (Eneje et al., 2001:725).

The overall values of hue angle as affected by particle sizes were 75.43, 76.66 and 75.55 they were significantly ($p < 0.05$) different for normal, coarse, and fine powder respectively. Umqombothi produced with coarse particle size had a greater hue angle than fine powder and normal particle sizes respectively. The trend can be justified by what was reported by Pahl et al., (2016:120), stating that the temperature, oxidation of polyphenols, and grist material, influenced the colour of the wort as well as during processing steps. The hue angles of the samples further suggested that the Umqombothi is dominated by a yellowish colour as they are close to a hue-angle of 90^o, which represents pure yellowness.

Table 4.9: The effect of particle sizes on hue angle (h^o) values of Umqombothi

	Hue angle (h^o)		
Sampling stages	**Normal**	**Coarse**	**Fine Powder**
Before first fermentation (BFF)	$74.19 \pm 0.00^{a,c}$	76.68 ± 0.00^{a}	77.79 ± 0.00^{a}
After first fermentation (AFF)	$75.30 \pm 0.56^{b,e}$	81.87 ± 0.00^{b}	82.90 ± 0.00^{b}
Before second fermentation (BSF)	$74.52 \pm 0.00^{c,ae}$	74.44 ± 0.00^{c}	69.55 ± 0.00^{c}
After second fermentation (ASF)	78.19 ± 0.18^{d}	77.11 ± 1.79^{a}	73.94 ± 0.00^{d}
Final product (FP)	$74.93 \pm 0.09^{e,cb}$	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}

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

The colour differences (ΔE) between Umqombothi samples with the different particle sizes ranged from 0.68 to 10.58. Colour difference (ΔE) < 1 can be defined as a "not noticeable difference", where the observer does not notice the difference. Colour difference (ΔE = 1) can be a just noticeable difference (JND). The colour difference between 4-and 8 is perceivable

but accepted (Murevanhema & Jideani, 2015), which entails that an observer notices the colour difference and is considered acceptable.

The colour difference between Umqombothi samples in normal and coarse particle sizes was not noticeable, as (ΔE) < 1 shows (0.68). The difference in colour between normal and powder was noticeable, but acceptable because the colour difference was 5.36. The difference in colour between coarse and powder was 10.58, and it was unacceptable. The appearance of Umqombothi produced with different particle sizes are presented in figure 4.5. When Umqombothi was made from normal particle size, it was darker than when Umqombothi was made from coarse and fine powder particle sizes, respectively.

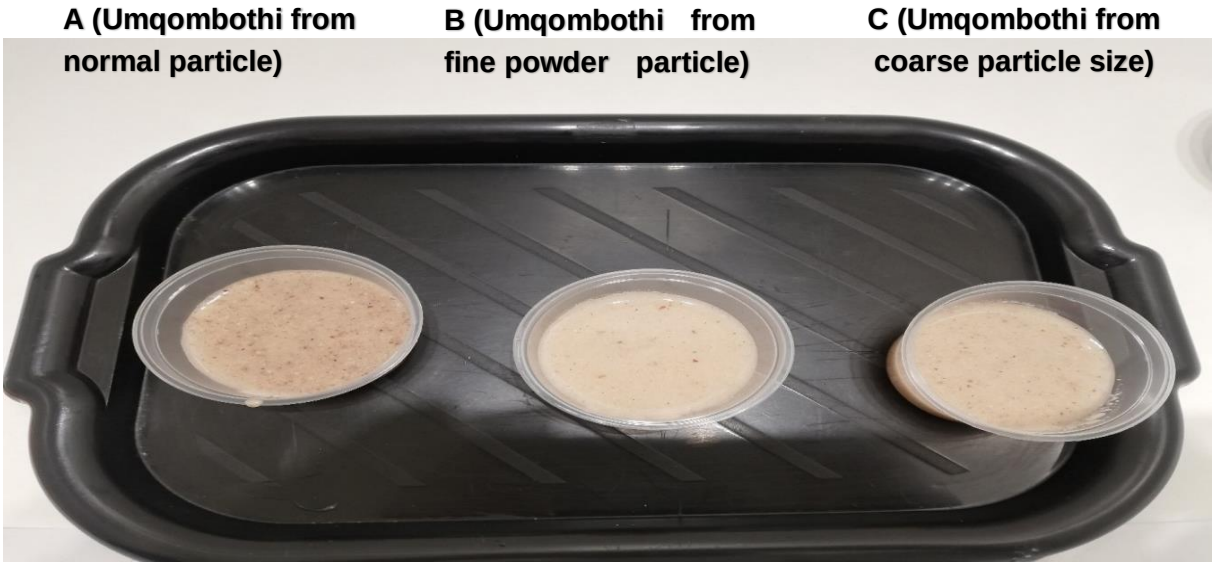

Figure 4.4: Appearance of Umqombothi produced with different particle sizes A (with normal particle size), B (with coarse particle size) and C (with fine powder particle size)

4.9.2.2 Effect of particle sizes on percentage syneresis (%STS) of Umqombothi during the production process

In the field of food science, syneresis is the process of extracting or expelling a liquid, such 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 (Samson A. et al., 2016). The %syneresis of Umqombothi from different particle sizes, at different sampling stages are presented in Table 4.10. The syneresis of sampling stages of BFF, AFF, BSF, ASF and FP ranged from 0.00-95.81, 0.00-93.28 and 0.00-91.36% for normal, coarse, and fine powder particle sizes, respectively. There was a significant ($p < 0.05$) difference of syneresis between all sampling stages for normal, coarse, and fine powder. There was a significant ($p < 0.05$) decrease in syneresis, from stages BFF to FP. Cooking (BSF), resulted into a significant ($p <$

0.05) decrease, while sieving resulted into significant (p < 0.05) increase in syneresis of Umqombothi. This is due to the removal of solids. The overall particle sizes values of syneresis were 51.19, 51.60, 52.36% and they were significantly (p < 0.05) different for normal, coarse, and fine powder. The normal particle size had the significant (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 (Hlangwani et al., 2020:10; Schaepdrijver, 2004:601).

Table 4.10: The effect of particle sizes %STS values of Umqombothi

	Syneresis (%)		
Sampling stages	**Normal**	**Coarse**	**Fine Powder**
Before first fermentation (BFF)	95.81 ± 0.01[a]	93.28 ± 0.01[a]	91.36 ± 0.00[a]
After first fermentation (AFF)	89.29 ± 0.02[b]	95.00 ± 0.01[b]	90.78 ± 0.01[b]
Before second fermentation (BSF)	0.00 ± 0.00[c]	0.00 ± 0.00c	0.00 ± 0.00[c]
After second fermentation (ASF)	28.29 ± 0.01[d]	25.38 ± 1.73[d]	33.45 ± 0.01[d]
Final product (FP)	42.57 ± 0.02[e]	44.36 ± 0.02[e]	46.19 ± 0.02[e]
Overall particle sizes	51.19 ± 37.78[a]	51.60 ± 38.80[b]	52.36 ± 36.26[c]

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

4.9.2.3 Effect of particle sizes on the viscosity of Umqombothi during the production process

The effect of particle size on Umqombothi (viscosity vs time)

The apparent viscosity vs time of normal, coarse and powder particle size Umqombothi, before and after fermentation are presented in Figures 4.6. The AFF viscosity observed for the normal particle size Umqombothi (A) was initially 400.0 MPa.s^{-1} at approximately 0 s and gradually increased to 800.0 MPa.s^{-1} over 15 s. The same effect was observed (BFF), which was initially -300.0 MPa.s^{-1} and gradually increased to 0.0 MPa.s^{-1} over 15 s (A). Coarse particle size Umqombothi (ASF) was initially 100.0 MPa.s^{-1} gradually increased to 200.0 MPa.s^{-1} over 15 s. BFF, it was initially 0.0 MPa.s^{-1} at approximately 0 s and gradually increased to 10.0 MPa.s^{-1} at 17 s indicated in (B). Fine powder particle size Umqombothi (ASF) was initially 150.0 MPa.s^{-1} at approximately 0 s and gradually increased to 220.0 MPa.s^{-1} at 17 s. The same effect was observed for BFF. It was initially 5.0 MPa.s^{-1} at approximately 0 s and gradually increased to 10.0 MPa.s^{-1} at 17 s (C). Normal, coarse, and powder samples of Umqombothi had a thicker viscosity before fermentation than after fermentation, respectively. The viscous properties in

relation to 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 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 (Jideani et al., 2021).

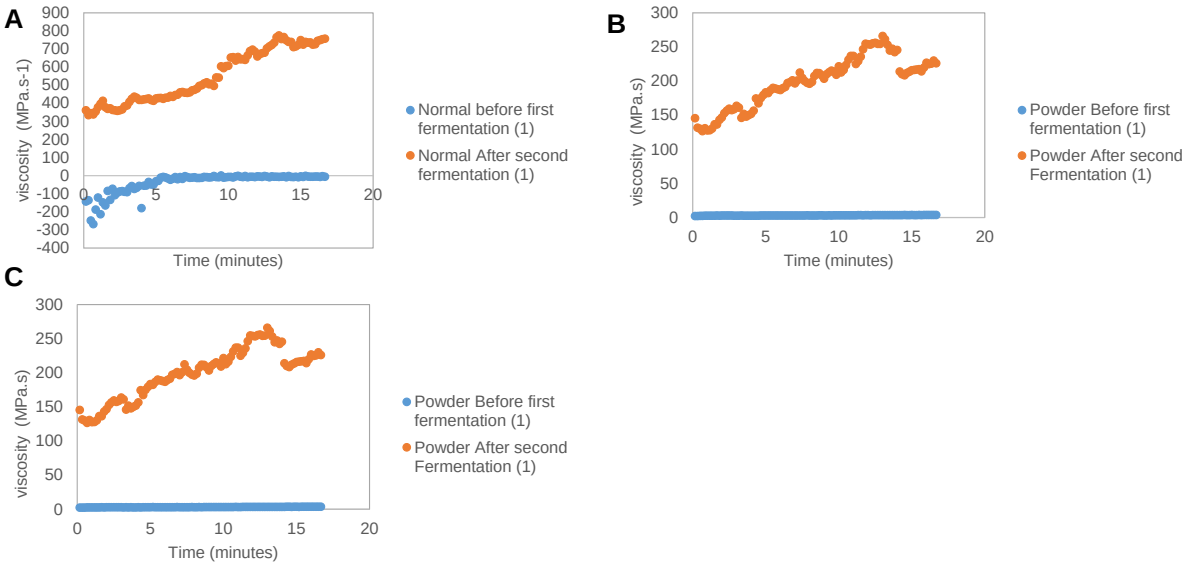

Figure 4.5: Apparent viscosity of (A) Normal particle, (B) Coarse particle and (C) Fine powder particle size of Umqombothi as a function of time at a constant shear rate (500 s^{-1}). Before first fermentation (BFF), After second fermentation (ASF)

The effect of particle size on Umqombothi (viscosity vs temperature)

The apparent viscosity as a function of fermentation temperature for normal, coarse, and fine powder particle size Umqombothi (BFF and ASF) is presented in Figures 4.7. Normal particle size, ASF was initially 800.0 MPa.s^{-1} at approximately 0ºC and gradually decreased to 200.0 MPa.s^{-1} at 25ºC (A). The same effect was observed BFF. It was initially 0.0 MPa.s^{-1} at approximately 0ºC and gradually decreased to -300.0 MPa.s^{-1} at 25ºC. The coarse particle size Umqombothi (ASF) was initially 200.0 MPa.s^{-1} at approximately 0ºC and gradually decreased to 100.0 MPa.s^{-1} at 25ºC (B). The same effect was observed for BFF. It was initially 8.0 MPa.s^{-1} at approximately 3ºC and gradually decreased to 4.0 MPa.s^{-1} at 25ºC. The fine powder particle size Umqombothi (ASF) was initially 240.0 MPa.s^{-1} at approximately 3˚C and gradually decreased to 100.0 MPa.s^{-1} at 25ºC (C). The same effect was observed BFF. It was initially 10.0 MPa.s^{-1} at approximately 4ºC and gradually decreased to 5.0 MPa.s-1 at 25ºC indicated in**.**

The viscous properties of Umqombothi (BFF and ASF) showed a decreasing effect when the temperature was increased at a constant shear rate (500 s^{-1}). The above-observed trend reveals the shear thinning pseudoplastic behaviour of Umqombothi. The absence of shear and temperature results in higher viscosity, and its application results in decreased viscosity. Before fermentation, the viscosity of all beer samples (normal, coarse, and fine powder particle sizes) for viscosity vs temperature were higher than 200 $MPa.s^{-1}$. As much as the viscosity of all beer samples decreased, they did not go below 100 $MPa.s^{-1}$. The cereals' viscosity decreases due to LAB's conversion of starch into simpler sugars during fermentation. The pH, the type of microorganisms, and whether those microorganisms include amylase enzymes that hydrolyse starch into dextrin and sugars all impact the beverage's viscosity (Jideani et al., 2021:18; Blšáková et al., 2022:7). The viscosity and nutritional value of the solution improved in various ways due to the LAB's breakdown of the starch (Jideani et al., 2021:18). These findings concur with those of (Hayta et al., 2001:336), who noted a reduction in viscosity following fermentation of a conventional fermented beverage (Boza) at 20°C. According to Hayta et al., (2001:336), heating reduces viscosity by decreasing molecular entanglement and stabilising 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 pseudoplastic behaviour concerning temperature as indicated by the flow behaviour index and consistency index values. The processing needs for the synthesis of Umqombothi may also depend on biochemical changes during fermentation, such as pH and changes in water-soluble proteins.

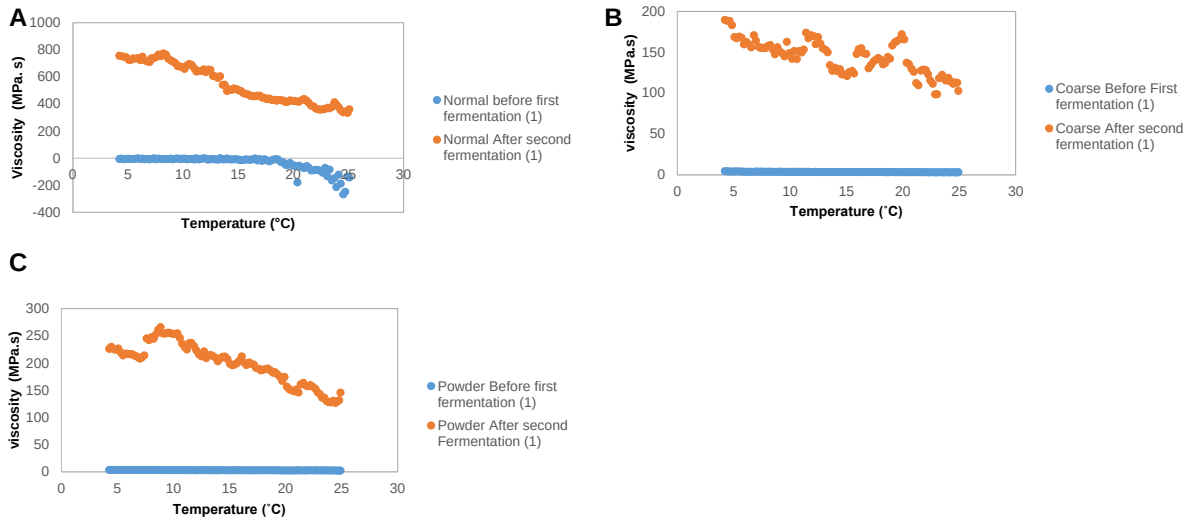

Figure 4.6: Apparent viscosity of the (A) Normal, (B) Coarse and (C) Fine powder particle sizes Umqombothi as a function of temperature at a constant shear rate (500 s^{-1}). Before first fermentation (BFF), After second fermentation (ASF)

4.9.3 Microbial population in Umqombothi

The LAB counts during Umqombothi production for different particle sizes are displayed in Table 4.11. Lactic acid bacteria present at sampling stages BFF, AFF, BSF, ASF and FP ranged from 0.00-8.16, 0.00-7.93 and 0.00-8.03 log cfu/ml for normal, coarse, and fine powder particle sizes, respectively see Figure 4.23. There was no significant difference between AFF-ASF, sampling stages of normal particle sizes. There was significant ($p < 0.05$) difference, between all sampling stages for coarse, and fine powder particle sizes. The LAB counts increased significantly ($p < 0.05$) after fermentation stages for normal, coarse, and fine powder particle sizes. Lactic acid bacteria is the most dominant microorganism during fermentation in sorghum (Hlangwani et al., 2020:9), with fewer occurrences and reports of yeast and fungi. The environment in which LAB thrives is rich in protein, sugar, vitamins, nucleotides, and lipids (Hlangwani et al., 2020:10), which could explain their predominance in the sorghum microflora. LAB was not detected at the BSF sampling stages for normal, coarse, and fine powder. This could be due to cooking temperature and time during mashing, which do not favour LAB growth. The LAB increased ($p < 0.05$) significantly between BFF and FP sampling stage for all three particle sizes. According to Hlangwani et al., (2020:9), this category of microorganisms is exploitative competitors that impede other microorganisms by rapidly utilizing plentiful glucose and accumulating acetic and lactic acid. The FP sampling stage LAB values of 8.16, 7.11 and 5.91 log cfu/ml for normal, coarse, and powder particle sizes, respectively, are similar to the LAB values of 8.56, 7.96 and 7.82 log cfu/ml reported by Katongole, (2008:66) in indigenous fermented maize. However, the results are higher than those reported by (Attchelouwa et al., 2017b:4), of 4.94 log cfu/ml.

The LAB levels of the overall particle sizes of 5.77, 5.53 and 5.36 log cfu/ml for normal, 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 Katongole, (2008:66). However, the levels are higher than those reported by Attchelouwa et al., (2017:4) of 4.94 log cfu/ml. The decrease of non-detectable microorganisms at BSF sampling stages for all particle sizes, could be due to cooking time and temperature. Cooking reduces the number of bacteria (Russel, 2003:11). Heat treatment and fermentation improve the beverage's taste, odour, and digestibility. During cooking, starch is converted to fermentable sugars, amino acids, and vitamins, which aids in the development of LAB and yeast to produce flavour, and fragrance and preserve the Umqombothi beverage sensory quality profile. They also extend shelf life by preventing bacteria, yeast, and mould growth that can cause spoiling and poisoning (Adekoya et al., 2018:22; Bayoï & Etoa, 2021:1). The addition of sorghum malt before second fermentation increased the product's total microbial load. The microorganisms present during the manufacturing of Umqombothi beer, have shown the same trend (Bayoï & Etoa, 2021:76).

Table 4.11: The effect of particle sizes on lactic acid bacteria (LAB) counts of Umqombothi

	LAB (cfu/ml)		
Sampling stage	**Normal**	**Coarse**	**Fine powder**
Before first fermentation (BFF)	5.02 ± 0.08[a]	4.99 ± 0.01[a]	5.09 ± 0.02[a]
After first fermentation (AFF)	7.86 ± 0.02[b]	7.93 ± 0.01[b]	8.03 ± 0.07[b]
Before second fermentation (BSF)	0.00 ±0.00[c]	0.00 ± 0.00[c]	0.00 ± 0.00[c]
After second fermentation (ASF)	7.83 ±0.03[b]	7.63 ± 0.05[d]	7.77 ± 0.04[d]
Final product (FP)	8.16 ±0.11[e]	7.11 ± 0.02[e]	5.91 ± 0.09[e]
Overall particle sizes	5.77 ± 3.21[a]	5.53 ± 3.05[b]	5.36 ± 3.00[c]

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

It is no surprise that LAB is one of the most common bacteria involved in sorghum fermentation, with LAB being the primary carrier. The TVCs counts during Umqombothi for different particle sizes are displayed in Table 4.12. The TVC of sampling stages BFF, AFF, BSF, ASF and FP ranged from 0.00-8.39, 0.00-7.94 and 0.00-8.04 log cfu/ml for normal, coarse, and fine powder particle sizes, respectively see Figure 4.24. There was a significant ($p < 0.05$) difference in TVC between all sampling stages for normal, coarse, and fine powder particle size, respectively. There was a significant increase ($p < 0.05$) in TVC between BFF-FP for all three particle sizes. TVCs were not detected in the BSF sampling stages for normal, coarse, and fine powder particle sizes. The increase of TVCs before the second fermentation stage is caused by the addition of sorghum malt and the starter culture, while the decrease in the final product for coarse and fine powder particle seizes was caused by the removal of solids during sieving. There was a significant ($p < 0.05$) difference in the TVC levels of the overall particle sizes for normal, coarse, and powder particle sizes and normal particle size had the highest TVC value. According to Katongole, (2008:2 and Hlangwani et al., (2020:5) The current study found that combining cooking and fermentation, as done with Umqombothi, increases the nutritious quality of sorghum while lowering the concentration of anti-nutritional components to a safe level. The high moisture content, the presence of nutrition, and contaminated microorganisms from the raw material contributed to the significant increase in TVC in the final product (Adekoya et al., 2019:22). The inclusion of tainted sorghum malt after heating (BSF) would have likely resulted in a massive increase in the TVC in the final product. The TVCs of the overall particle sizes of 5.95, 5.58 and 5.38 log cfu/ml for normal, coarse, and powder particle sizes, respectively, are lower than the TVC values of 8.66 cfu/ml reported on a sorghum beer final product by Attchelouwa et al., (2017:4).

Table 4.12: The effect of particle sizes on total viable counts (TVC) of Umqombothi

	Total viable count (cfu/ml)		
Sampling stage	**Normal**	**Coarse**	**Fine powder**
Before first fermentation (BFF)	5.62 ±0.11[a]	5.10 ± 0.03[a]	5.37 ± 0.04[a]
After first fermentation (AFF)	7.95 ± 0.01[b]	7.94 ± 0.02[b]	8.04 ± 0.05[b]
Before second fermentation (BSF)	0.00 ±0.00[c]	0.00 ± 0.00[c]	0.00 ± 0.00[c]
After second fermentation (ASF)	7.79 ± 0.01[d]	7.61 ± 0.03[d]	7.71 ± 0.03[d]
Final product (FP)	8.39 ±0.14[e]	7.26 ± 0.04[e]	5.81 ± 0.03[e]
Overall particle sizes	5.95 ± 3.24[a]	5.58 ± 3.07[b]	5.38 ± 2.99[c]

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

Adekoya et al., (2019:4) reported that the total aerobic count of samples ranged from 5.7 to 10.8 log cfu/ml/ in final product of Umqombothi. The yeast counts during Umqombothi for different particle sizes are displayed in Table 4.13. The yeast count of sampling stages BFF, AFF, BSF, ASF and FP ranged from 0.00-7.89, 0.00- 7.79 and 0.00-7.91 log cfu/ml for normal, coarse, and fine powder particle sizes, respectively, see Figure 4.25. There was a significant ($p < 0.05$) difference in the yeast count between all sampling stages for normal, coarse, and fine powder particle sizes. The yeast increased ($p < 0.05$) significantly between BFF-FP and decreased ($p < 0.05$) significantly between ASF-FP sampling stages for all three particle sizes. Firstly, beer yeasts are extracted and re-used after the fermentation process to begin the following fermentation batch, a process called 'backslopping" (Gallone et al., 2018:148)'. The yeast reduction may be related to the elimination of solid particles during sieving. Alcoholic fermentation is one of the rocessses in the conventional process that mostly defines the end product's quality (Coulibaly et al., 2018:612). Yeast was not detected in the BSF sampling stages for normal, coarse, and fine powder particle sizes, this could be due to cooking temperature and time.

There was a significant ($p < 0.05$) difference in overall particle sizes yeast values 4.77, 4.93 and 5.18 log cfu/ml between normal, coarse, and powder particle sizes. The fine powder particle size had the highest yeast value. Due to the presence of LAB, the lower the alcohol concentration, pH, and the viscosity, the longer the fermentation was permitted to continue (Jideani et al., 2021:18). The overall particle sizes yeast values of 4.77, 4.93 and 5.18 log cfu/ml for normal, coarse, and powder particle sizes, respectively, are lower than the levels reported of Umqombothi final product by Katongole, (2008:66) of 6.52, 7.1 and 6.42 log cfu/ml and yeast values of 8.05 cfu/ml of Ivorian sorghum beer reported by Attchelouwa et al., (2017:4).

Table 4.13: The effect of particle sizes on yeast counts of Umqombothi

	Yeast (cfu/ml)		
Sampling stage	**Normal**	**Coarse**	**Fine powder**
Before First fermentation (BFF)	5.09 ±0.01[a]	5.09 ± 0.04[a]	5.25 ± 0.01[a]
After first fermentation (AFF)	7.56 ±0.03[b]	7.14 ± 0.01[b]	7.12 ± 0.02[b]
Before second fermentation (BSF)	0.00 ±0.00[c]	0.00 ± 0.00[c]	0.00 ± 0.00[c]
After second fermentation (ASF)	7.89 ±0.05[d]	7.79 ± 0.05[d]	7.91 ± 0.06[d]
Final product (FP)	3.3 ±0.12[e]	4.64 ± 0.05[e]	5.63 ± 0.03[e]
Overall particle sizes	4.77 ± 3.01[a]	4.93 ± 2.83[b]	5.18 ± 2.86[c]

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

Adekoya et al., (2018:26) reported a yeast count of 2.3 x 10^7 cfu/ml for 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 flavours (Cubillos et al., 2019:383). According to Hlangwani, et al., (2021:1), the environment in which yeast grows is rich in protein, sugar, vitamins, nucleotides, and lipids, which could explain their predominance in the sorghum microflora. Umqombothi is nutrient-rich, explaining why yeast numbers increased dramatically after fermentation (Katongole, 2008:59; Schaepdrijver, 2004:600). Fermentation normally causes enzyme activation, a decrease in pH, and increased microbial activity. This causes the breakdown of the substrate, boosting of the nutritional quality, thereby favouring yeast growth (Mensah, 1997:272).

The yeast counts obtained in this study confirm that yeast is one of the most dominant microorganisms in Umqombothi. Yeast counts also demonstrate the effect of particle sizes on Umqombothi. The fine powder and coarse particle sizes produced higher yeast counts than the normal particle size. This would impact the flavour of the final products. The role of a yeast strain in product quality is frequently underestimated, yet the specific strain used will significantly impact the beer character as a raw material (Cubillos et al., 2019:383). Brewing yeast directly impacts the character and quality of beer, depending on which type is used to make a particular style. Traditional fermented beverages could be a valuable supply of yeast strains for the brewing business. In addition to *S. cerevisiae*, many traditional fermented beer and other beverage production processes initiate spontaneously and often contain a combination of native yeast strains. Yeast is responsible for converting fermentable

carbohydrates into ethanol and creating many active flavour chemicals (Cubillos et al., 2019:389).

The mould counts during Umqombothi production for different particle sizes are displayed in Table 4.14. The mould count of sampling stages BFF, AFF, BSF, ASF and FP ranged from 0.00-4.39, 0.00-4.29, and 0.00-4.08 log cfu/ml for normal, coarse, and fine powder particle sizes, respectively, see Table 4.14. There were no moulds detectable in the BSF-ASF sampling stages for normal, coarse, and fine powder particle sizes, it could be that the fungi and its spores were destroyed during cooking. There was no significant difference, between BSF, ASF and FP sampling stage of the fine powder particle size. The mould counts decreased ($p < 0.05$) significantly to non-detected, between BFF-ASF sampling stage for all three particle sizes. The pH significantly ($p < 0.05$) decreased from BFF (6.03, 6.02, and 5.96) to FP (3.45, 3.46, and 3.49) see Figure 4.6, as the LAB counts increase from BFF (5.02, 4.99, and 5.09 log cfu/ml) to FP (8.16, 7.11, and 5.91 log cfu/ml Figure 4.24) see Figure 4.23, resulting in a significant ($p < 0.05$) decrease in mould counts to non-detected for normal, coarse, and fine powder particle sizes.

There was significant ($p < 0.05$) difference in the mould counts of the overall particle sizes 2.14, 1.85 and 1.48 log cfu/ml between normal, coarse, and powder particle sizes. The normal particle size exhibited the significantly ($p < 0.05$) highest mould counts. The unfavourable fermentation temperature and pH environment reduced moulds during the Umqombothi production process. This condition is the reason for the significant ($p < 0.05$) decrease in mould counts. The decrease in pH of the final product and the removal of solid particles also affected mould counts. The significant decrease of mould in Umqombothi at before second fermentation sampling stage corresponds with results obtained by Bayoï & Etoa, (2021:77) in the production of the white “mpedli” beer. Umqombothi is sensitive to quick degradation due to moulds, which could be one of the reasons it has a shelf-life of only 2-3 days (Blandino et al., 2003:539; Adekoya et al., 2018:22). The total coliforms counts during Umqombothi production for different particle sizes are displayed in Table 4.15. The total coliform count obtained at sampling stages BFF, AFF, BSF, ASF and FP ranged from 0.00-4.82, 0.00-4.86, and 0.00-5.16 log cfu/ml for normal, coarse, and fine powder particle sizes, respectively, see Figure 4.27. There was no significant difference, between BSF (0.00 log cfu/ml), ASF (0.00 log cfu/ml) and FP (0.00 log cfu/ml) sampling stage of normal particle size Umqombothi production process. This could be due to the significantly low pH at ASF and FP sampling stage (3.54-3.45) see figure 4.6. The total coliform counts decreased significantly ($p < 0.05$) during the BFF, AFF and ASF sampling stages for normal, coarse, and fine powder particle sizes, indicating that the conditions during the first and second fermentation were not ideal for coliforms to proliferate. There were no detectable coliforms present after cooking (BSF sampling stage) and remained absent at ASF sampling stage in the normal particle sizes.

Table 4.14: The effect of particle sizes on mould counts of Umqombothi

	Mould (cfu/ml)		
Sampling stage	**Normal**	**Coarse**	**Fine powder**
Before first fermentation (BFF)	4.06 ±0.10[a]	4.29 ± 0.09[a]	4.08 ± 0.04[a]
After first fermentation (AFF)	4.39 ±3.81[b]	1.67 ± 2.89[b]	3.33 ± 2.89[b]
Before second fermentation (BSF)	0.00 ±0.00[c]	0.00 ± 0.00[c]	0.00 ± 0.00[c]
After second fermentation (ASF)	0.00 ±0.00[c]	0.00 ± 0.00[c]	0.00 ± 0.00[c]
Final product (FP)	2.27 ±0.22[d]	3.30 ± 0.30[d]	0.00 ± 0.00[c]
Overall particle sizes	2.14 ± 2.43[a]	1.85 ± 2.10[a]	1.48 ± 2.19[a]

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

The total coliforms present in the overall sampling stages were BFF (4.95 log cfu/ml), AFF (3.98 log cfu/ml), BSF (0.00 log cfu/ml), ASF (2.51 log cfu/ml) and FP (0.00 log cfu/ml). The BFF, AFF and ASF overall sampling stages were significantly ($p < 0.05$) different. There were a significant ($p < 0.05$) differences in the total coliform counts of the overall particle sizes between normal, coarse, and fine powder particle sizes. The fine powder particle size had the significantly ($p < 0.05$) highest total coliforms value. This finding demonstrated that certain stages of heating and fermenting play a critical role in reducing coliform bacteria during the manufacturing process (Bayoï & Etoa, 2021:77). Coliforms are aerobic and facultative anaerobic bacteria that ferment lactose under gassy and acidic conditions and need temperatures ranging from 35 to 37^{o}C to proliferate (Hill, 2016:296). As water was one of the primary ingredients in the production of Umqombothi, an evaluation of total coliforms in Umqombothi was required (Eumann & Schaeberle, 2016:97). The results indicated that the heating and fermentation stages played an important role in controlling coliform bacteria during the production process (Sawadogo-Lingani et al., 2021:2).

Table 4.15: The effect of particle sizes on total coliforms counts of Umqombothi

	Total coliforms (cfu/ml)		
Sampling stages	**Normal**	**Coarse**	**Fine powder**
Before first fermentation (BFF)	4.82 ±0.04[a]	4.86 ± 0.06[a]	5.16 ± 0.05[a]
After first fermentation (AFF)	4.63 ±0.03[b]	3.52 ± 0.07[b]	3.79 ± 0.06[b]
Before second fermentation (BSF)	0.00 ±0.00[c]	0.00 ± 0.00[c]	0.00 ± 0.00[c]
After second fermentation (ASF)	0.00 ±0.00[c]	3.79 ± 0.02[d]	3.74 ± 0.03[d]
Final product (FP)	0.00 ±0.00[c]	0.00 ± 0.00[c]	0.00 ± 0.00[c]
Overall particle sizes	1.89 ± 2.39[a]	2.43 ± 2.11[b]	2.54 ± 2.21[c]

Mean value ± standard deviation of triplicate determination. Mean values in the same column followed by different letter are significantly ($p \leq 0.05$) different

4.9.4 Principal component of Umqombothi during production process

In an attempt to simplify the the interpretation of data, principal component analysis was applied to the data. As show in Figure 4.8, the measured variables of Umqombothi production process were reduce to three main components (F1, F2 and F3) by the PCA. F1 and F2 (Figure 4.8 A) explained 67.4%, F1 and F2 (Figure 4.8 B) explained 65.6% and F2 and F3 (Figure 4.8 C) explained 59% of the total data variance, respectively, for Umqombothi production process with different particle sizes. Figure 4.8 was created to explore the positive relationship between the different parameters studied during sampling stages. As show in Figure 4.8, the variances could be separated into two groups. The first group is compose of colour and second group is compose of microbes. Figure 4.8 (A), positive correlation was found between the F1 (chroma, yellowness, redness, alcohol, gravity) and F2 (mould, pH, LAB, yeast syneresis,TPC).

There was also a negative correlation between these compounds. Figure 4.8 (B), a positive corelation was found between the F1 (yellowness,redness, chroma, brix) and F3 (TPC, LAB,yeast). However, there was a negative corelation between these compounds and sampling stages of before second fermentation “after cooking” and after first fermentation and after second fermentation. Figure 4.8 (C), a positive correlation was found between the F2 (LAB, TPC, yeast, hue angle) and F3 (chroma, yellowness, redness, brix). There was no variance that was strongly corelated to before second fermentation (after cooking) sampling stage. As show in (Figure 4.8 D), the measured variable of Umqombothi with different particle sizes were reduce to two main component (F1 and F2) by PCA F1 and F2 explained 100% of total data variance. A positive corelation was found between F1 (syneresis yeast, gravity, brick coliforms), they were strongly corelated with fine powder particle size and F2 (TPC, LAB, mould, pH).

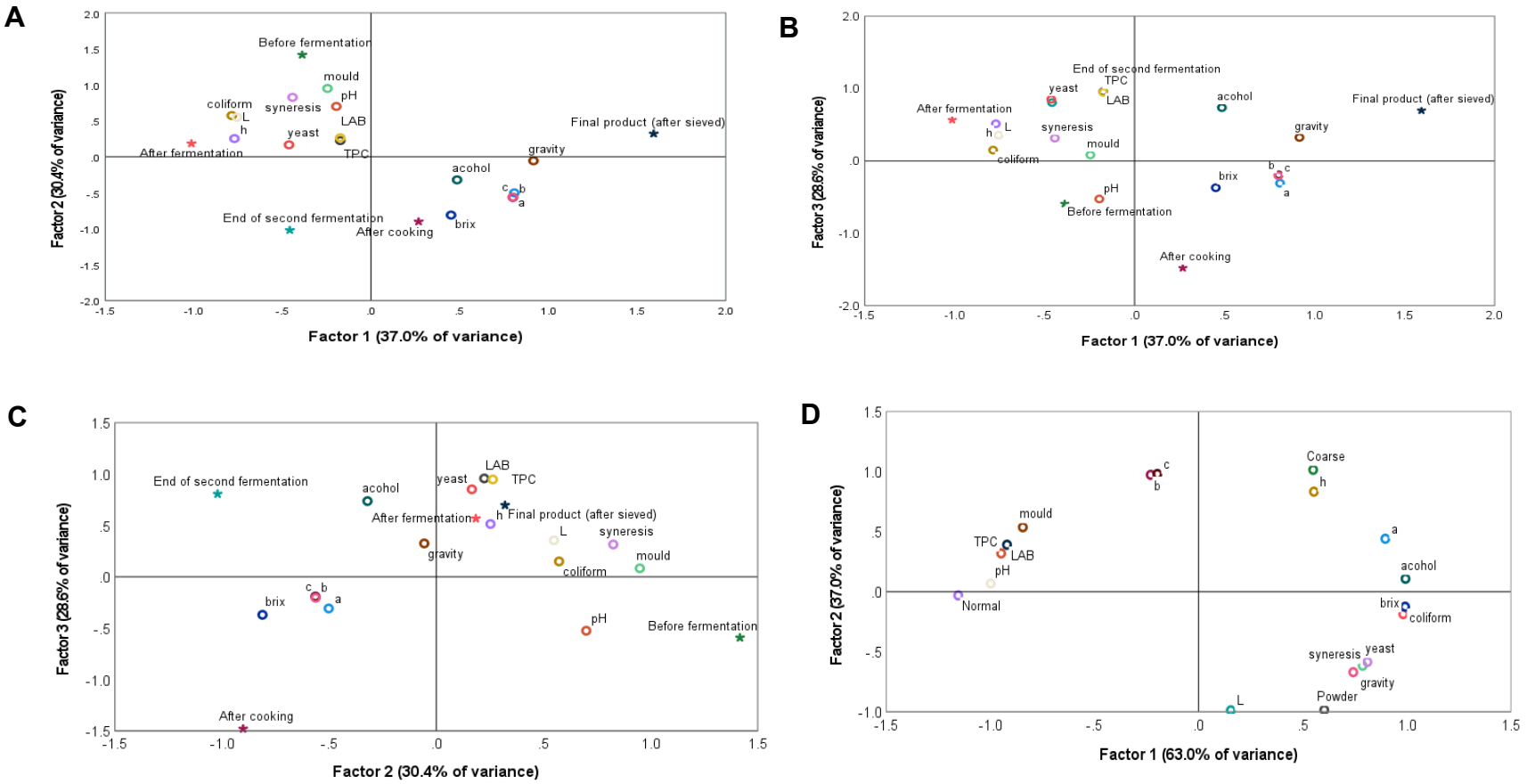

Figure 4.7: PCA plot base on Umqombothi production process. Sampling stages A, B and C. Particles si

4.9.5 Sensory characteristics of Umqombothi from three different particle sizes

The results of the sensory evaluation of Umqombothi produced using three different particle sizes are indicated in Table 4.16. There was no significant difference with regards to appearance and texture between Umqombothi prepared from normal, coarse, and fine powder particle sizes as it got the higher values of 3-Neither like or Dislike. Umqombothi from normal particles had 4-Like moderately higher rating for colour, aroma, taste and overall acceptability than 3-Neither like or Dislike rating for coarse and fine powder Umqombothi (final product), as much. The normal ingredients particle sizes, had an impact on the overall flavour profile of the beer but it was not significantly different, as much as normal particle size produces lower alcohol than the two particle sizes, coarse and fine powder. Umqombothi produced with normal particle sizes got better or higher scores from consumer sensory evaluation but the score were not significantly different.

Table 4.16: The Sensory evaluation of Umqombothi (final product) from different particle sizes

	Umqombothi from different particle sizes		
Sensory attributes	**Normal**	**Coarse**	**Fine powder**
Appearance	3.82 ± 0.89^{a}	3.76 ± 1.21^{a}	3.66 ± 1.19^{a}
Colour	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}

Mean value ± standard deviation of triplicate determination. Mean values in the same column, followed by different letter are significantly ($p \leq 0.05$) different

4.9.6 Sensory characteristics of laboratory Umqombothi and Township Umqombothi

The study compared the sensory attributes of Umqombothi produced in the laboratory with 30-30^{o}C fermentation temperatures and coarse particle sizes to traditional Umqombothi produced in the township of Mbekweni (Langabuya) and New-Rest (Ezimbacwini) in Cape Town, Paarl under Drakenstein Municipality see (Table 4.17 and Figure 4.9. Table 4.17) presents the percentage (%) overall acceptability according to the beverage ratings of Umqombothi produced in the laboratory and in the townships of Langabuya and Ezimbacwini. The panel consisted of approximately 34.7% males, 65.3% females, 85.7% blacks, 12.2% coloured, and 2% whites. Additionally 39.6% were CPUT staff members, 60.4% student, 10% International

students, and South African 90%. Sixty-nine percent (69%) were under 30 years old, 24.5% were between 30-39 years old, and 14.3% were over 40 years old.

The appearance distributions for Laboratory, Langabuya, and Ezimbacwini Umqombothi are fairly symmetrical, with Laboratory skewing upwards and Ezimbacwini skewing downwards. On the higher end, Ezimbacwini Umqombothi is more consistent. The mendian consumer panelist for all three Umqombothi samples was around 4, while the interquartile for laboratory was around 3, which was better than Langabuya and Ezimbacwini. The middle 50% spread was wider in laboratory Umqombothi. Ezimbacwini Umqombothi had a range of 3, while Laboratory and Langabuya Umqombothi had a range of 4. Umqombothi laboratory has the greatest overall spread. Laboratory and Ezimbacwini Umqombothi have fairly symmetric colour distributions, whereas Langabuya Umqombothi is skewed upwards. Langabuya Umqombothi has a higher consistency on the upper end, whereas Laboratory and Ezimbacwini Umqombothi have a larger colour variation but have the same median 4 as Langabuya Umqombothi. The interquartile for Laboratory and Ezimbacwini Umqombothi was 2, which was higher than the interquartile for Langabuya Umqombothi. Therefore, the spread of the middle 50% was large in Laboratory and Ezimbacwini Umqombothi. Ezimbacwini and Laboratory had a colour range of 4, while Langabuya Umqombothi had a colour range of 2. Ezimbacwini Umqombothi and Overal Laboratory had a large spread.

The aroma median for Laboratory Umqombothi was 3, while the aroma median for Langabuya and Ezimbacwini Umqombothi was 4. Laboratory and Langabuya Umqombothi had aroma interquartiles of 2, while Ezimbacwini had an aroma interquartile of 1. As a result, the dispersion of the middle 50% was greater in Laboratory and Langabuya Umqombothi. The scent range of Laboratory and Langabuya Umqombothi was 4 based on the complete aroma data set. As a result, Laboratory and Langabuya Umqombothi have the most spread. Ezimbacwini Umqombothi was constant 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 Laboratory and Langabuya had a tatse interquartile of 3. The middle 50% dispersion was wider in Laboratory and Langabuya Umqombothi. Overall laboratory and Langabuya have a wider 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 data set, Laboratory, Langabuya, and Ezimbacwini Umqombothi all had the same spread, same spread in the middle 50%, and same overall spread. The skew in Laboratory and Ezimbacwini was downward. Langabuya and Ezimbacwini Umqombothi are consistent to the middle, while Laboratory and Ezimbacwini Umqombothi have a bigger viriation of overall acceptance. The total acceptability median for Umqombothi samples from Laboratory, Langabuya, and Ezimbacwini was 4. The overall acceptance range for Laboratory and Ezimbacwini Umqombothi was 3, whereas Langabuya Umqombothi was 4. Laboratory and Ezimbacwini Umqombothi had the greatest spread

throughout the full data set. Langabuya Umqombothi had a wider middle 50% spread of 2 than Laboratory and Ezimbacwini Umqombothi. Langabuya has the widest range of overall acceptability. As compared to Langabuya and Ezimbacwini Umqombothi, Laboratory Umqombothi exhibits the most variety in all sensory qualities, as well as the highest median, range, and spread in the middle 50%.

The variations in Umqombothi could be attributed to the varying amounts of ingredients used, cooking temperature and time, fermentation temperature and time, hygienic utensils used, and 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 (Atter et al., 2017:352; Cadenas et al., 2021:12).

Table 4.17: Demography of panellists

Item		Frequency (%)
Gender		
	male	34.7
	Female	65.3
Race		
	Blacks	85.7
	Coloured	12.2
	Whites	2
Staff or student	Staff	39.6
	Student	60.4
International		
	Yes	10
	No	90
Age		
	< 20-29	69
	30-39	24.5
	40 & above	14.3

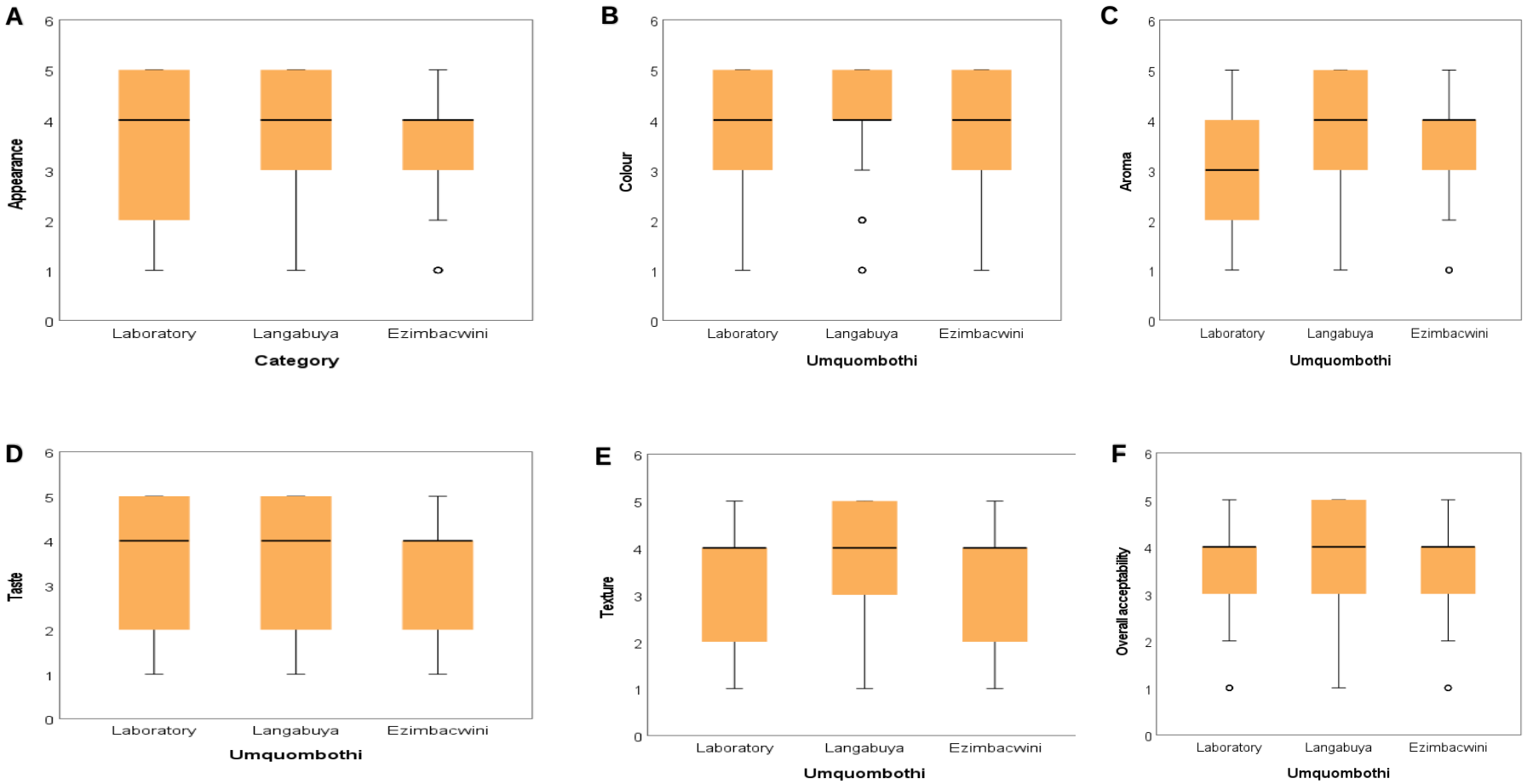

Figure 4.8: The distribution of sensory attributes. A-appearance, B-colour, C-aroma, D-taste, E-texture and F-overall acceptability

Table 4.18: The Sensory evaluation of Umqombothi (final product) from different locations

	Umqombothi Kruskal ranks		
Attribute	**Laboratory**	**Langabuya**	**Ezimbacwini (New-Rest)**
Appearance	74.47[a]	81.49[a]	70.54[a]
Colour	72.94[a]	80.26[a]	73.30[a]
Aroma	65.29[a]	81.54[a]	79.67[a]
Taste	77.22[a]	78.51[a]	70.77[a]
Texture	69.49[a]	82.74[a]	74.27[a]
Overall acceptability	67.26[a]	85.00[a]	74.24[a]

Mean value ± standard deviation of triplicate determination. Mean values in the same column, followed by different letter are significantly ($p \leq 0.05$) different

4.10 Conclusion

In conclusion, the present study shows that different particle sizes of Umqombothi ingredients do affect the final product chemically, microbiologically, and physically. Coarse particle size produced Umqombothi with the better overall quality, than normal and fine powder particles sizes Umqombothi. The particle sizes futher elaborate that the overall quality of Umqombothi can be inproved, without changing the formulation of the beverage but by improving the the production process. Coarse particle size Umqombothi was compared with traditionally prepared Umqombothi from the township. Laboratory Umqombothi has the biggest variation in all sensory attributes and the highest median, range and the largest spread in the middle 50%, as compared to Langabuya and Ezimbacwini Umqombothi. As the production of this beverage has important implications for the food system and economy of the country, further studies are required to find the compounds which mainly determine the acceptability or rejection of the product. Developingbetter preservation strategy using inoculation with pure yeast and LAB culture.

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CHAPTER 5

GENERAL SUMMARY AND CONCLUSIONS

1. Effect of particle size, lactic acid bacteria (LAB) and yeast strains on the stability and sensory characteristics of Umqombothi. The following objectives were identified in this study.
2. Establish the optimal fermentation time and temperature to produce Umqombothi.

   Establish the optimal ingredient particle size to produce Umqombothi.
3. Characterize and compare microbial, physicochemical, and stability (syneresis and viscosity) of optimized Umqombothi.
4. Evaluate consumer acceptability of the laboratory optimized Umqombothi against Umqombothi produced in townships by using traditional methods.

The first objective was achieved as Umqombothi was successfully produced using the cooking process of 60^{o}C for 40 minutes and employing the double spontaneous fermentation process at three different combination temperatures (25-30^{o}C, 30-25^{o}C and 30-30^{o}C). The chemical, physical and microbiological properties of Umqombothi were subsequently determined. Umqombothi was characterised with regard to pH, which is an important feature for traditional beers as high pH facilitates microbial growth and affects the product's shelf life and organoleptic properties (Hlangwani et al., 2020:11). Furthermore, the pH, lactic acid bacteria (LAB) and yeast counts of Umqombothi were comparable to various other traditional beers. Lactic acid bacteria cause the low pH in Umqombothi by producing lactic acid and acetic acid during fermentation. Total soluble solid (TSS) and Specific gravity (SG) are economically and commercially important properties of beer. Hydrolysis of starch into fermentable sugars is achieved during cooking, however, complete gelatinization of starch is not permitted. Starch in African beer acts as a source of sugar, and thickener and suspending agent (Lyumugabe et al., 2012:513). The colour of Umqombothi in this study concurs with other studies and people perceptions. All the colour attributes were positive (a=red and b=yellow). The small particles of cereal grain, starch granule and suspended yeast, cause Africa beers to be opaque and high in viscosity. This is to be expected, given that most of the maize used in traditional beer is yellow maize and sorghum malt is red. The hypothesis that, the optimize fermentation time and temperature of Umqombothi will improve the sensory characteristics and will be acceptable to the consumer, was accepted.

The second objective was achieved by establishing the optimal ingredient particle size to produce Umqombothi. This involved investigating the effect of normal, coarse and fine powder particle sizes on Umqombothi. The high alcohol content and low pH of Umqombothi

indicate its potential to have a longer shelf life. Low pH and high alcohol control microbial growth, affecting the product's shelf life and organoleptic properties (Hlangwani et al., 2020:10). The pH and acidity of Umqombothi indicated its potential of being a fermented drink. The colour of Umqombothi position the product as normal traditional Umqombothi that people are familiar with. As the particle size decrease, the syneresis, yeast counts, TSS, colour increases, while LAB decrease, and indicate the effect of particle sizes. The absence or decrease of coliforms and *Enterobacteriaceae* in Umqombothi revealed its potential long-life storage. Therefore, the optimal ingredient particle sizes (coarse particle size) to produce Umqombothi revealed its potential as a traditional beverage. The hypothesis that, reduced ingredient particle sizes will improve the stability of Umqombothi was accepted.

The third objective was to characterize and compare microbial, physicochemical, and stability (syneresis and viscosity) of optimized Umqombothi. Umqombothi prepared from all particle sizes exhibited a low pH, high total soluble solids, acceptable alcohol content and significant pattern of syneresis which is beneficial for it shelf life and appearance to the consumer. The fourth objective was evaluating consumer acceptability of the optimized Umqombothi. Two samples of traditionally prepared Umqombothi were collected from private households in Cape Town, in the townships of Paarl which are Mbekweni and NewRest, under the Drakenstein Municipality. A sensory evaluation was conducted on Umqombothi samples and the laboratory optimized Umqombothi.

5.1 The following conclusions can therefore be drawn from this study:

This study focused on microbial changes that occurred during the processing of Umqombothi in South Africa's Western. The findings revealed that, even though Umqombothi sorghum beverages are produced using various processes, many factors promote contamination, including raw material and poor quality of water used in beer production, equipment used during manufacturing, the technique used to cool the boiled wort, and environmental sanitation. It was also discovered that proper raw material handling, heat treatment execution, and fermentation can all be considered critical points that must be checked and optimized to control microbial contamination. Because of the potential for spoilage and shortening of shelf life, the bacterial load in both the malt and ready-to-serve product should be carefully monitored. These findings provide useful information on the microflora found in Umqombothi samples and the microbial changes that occur during some production processes.

The data collected may also allow local producers to predict potential contamination hazards and identify true critical points. This could help to improve the safety of indigenous sorghum-based beers produced in South Africa's Western and Eastern provinces. As a result, processors and sellers should be trained in good manufacturing and hygiene practices, such as keeping raw materials in a proper dry place, pre-boiling water and keeping it in covered clean containers for cooling before use and using proper utensils and equipment during and

after production. Findings in this study lay the foundation for a better understanding of the microbiological, chemical, physical, and sensory qualities of Umqombothi indigenous beers produced in South Africa's Western Cape provinces.

1.First and second fermentation temperatures of (30^{o}C-30^{o}C) for 24 hours can be used to produce Umqombothi that was acceptable to the consumers.
2.Umqombothi can be produced from maize meal, maize malt, and sorghum malt, cooked at 60^{o}C for 40 minutes.
3.Umqombothi has chemical, microbiological, and physical properties which are comparable to other traditional beers.
4.Umqombothi produced from coarse particle sizes was acceptable to consumers
5.Township Umqombothi was mostly preferred by consumer during sensory evaluation

5.2 Recommendations

There are many fermented foods around the world, which are produced when a variety of microbes metabolize a range of various substrates to produce foods with distinctive and alluring qualities. The molecular and microbiological underpinnings of the fermentation processes in many of these foods are poorly described. This study emphasizes how making native grain fermented beverages in Southern Africa is still mostly a traditional art that is characterized by inadequate cleanliness, variable presentation of quality, and short shelf life. The creation of these native beverages typically relies on the accidental or spontaneous inoculation with LAB. It is therefore highly advised to strengthen the control of such fermentations and product features, including, but not limited to, the use of purified starter cultures with suitable technological characteristics. It is likely that the fundamental microbiological studies along with the appropriate technological advancements will be sufficient to accomplish these goals. The objective here would be to further improve reliability and product quality through starter culture performance optimization and to remove those factors that interfere with the fermentation process. In terms of process variables like preparation time, the types of cereals utilized, and the mix of these cereals, indigenous cereal fermentations in Southern Africa are slightly different, although it is correct to state that they are essentially comparable.

There will surely be a need in future to produce these foods in settings where quality and safety can be ensured, therefore greater management of fermentations and product characteristics is strongly advised to retain and sustain African indigenous fermented foods and beverages. To provide more dependable and predictable fermentation processes, this calls for a deeper understanding of the microorganisms involved, both in terms of type and their specialized activity.

APPENDICES

APPENDIX A: Physicochemical and microbiological changes during two-stage fermentation production of Umqombothi Abstract accepted for poster presentation at SAAFoST Congress 2023 on 28th to 30th August 2023.

Appendix B: Under review research paper titled; Physicochemical and microbiological changes during two-stage fermentation production of Umqombothi.

Appendix C: Approved Ethics Clearance