Evaluation of the microbiological, nutritive and health properties of a millet-based fermented beverage

by

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DISSERTATION

Submitted in fulfilment of the requirements for the degree of

Master of Science

in

Microbiology

in the

FACULTY OF SCIENCE AND AGRICULTURE

School of Molecular and Life Sciences

at the

UNIVERSITY OF LIMPOPO

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2024

DECLARATION

I declare that the dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Microbiology has not been previously submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

Signature:  08/04/2024

Ms Mogashoa D Date

DEDICATION

I dedicate this work and scholarly efforts to the God of Mount Zion. A special dedication to my mother who always encouraged, motivated, prayed, supported, and believed in me. I also dedicate this to my brothers, sisters, and siblings for always highlighting that everything is possible through prayer, perseverance, and dedication.

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation and thanks to God of Mount Zion for his love, protection, grace, mercy and for giving me strength and courage to complete my research work.

My sincere gratitude is extended to Professor Kgabo L.M Moganedi (Supervisor), Professor Elbert Jansen Van Rensburg (Co-Supervisor) and Dr Lesetja M Legodi (Co-Supervisor) for sharing scientific knowledge and guidance, being patient as well as trusting me with this project.

I thank the Council for Scientific and Industrial Research (CSIR) for providing extensive financial support and constantly offering support and mental wellness sessions.

I would also like to thank the indigenous knowledge team for volunteering as tasting panellist for the laboratory produced mageu.

My greatest gratitude goes to my family (The Mogashoa family) for their endless support and encouragement.

The University of Limpopo, Faculty of Science and Agriculture, School of Molecular and Life Sciences for giving me the opportunity to successfully conduct my research project.

All thanks to the Department of Biochemistry, Microbiology and Biotechnology for their services.

Lastly, I would like to thank Limpopo Agro-Food Technology Station (LATS) for assisting with mineral analysis and microbiological analysis, and Inqaba Biotechnology research team for helping with identification of unknown lactic acid bacteria (LAB) isolates.

Inspirational bible verse

1 Thessalonians 4 verse 11 – 12 “And make it your ambition to lead a quiet life. You should mind your own business and work with your own hands, just as we told you. So that your daily life may win the respect of outsiders and so that you will not be dependent on anybody”.

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ABSTRACT

This study explored the potential of millet as a mainstream food source, emphasising its exceptional nutritional composition that promotes good health. The research focused on millet-based mageu, analysing two formulations: one with 90% millet flour and the other with 50%, over an 8-week storage period. The assessment included lactic acid bacteria (LAB), total aerobic bacteria, *Escherichia coli*, and moulds/yeasts. LAB were examined for probiotic traits like acid and bile tolerance, antibiotic susceptibility, and antioxidant activity. Chemical profile, nutritional, and sensory characteristics of mageu products were also evaluated. Viable LAB cells in both mageu formulations ranged from 193×10^4 to 24×10^6 CFU/mL, surpassing total aerobic bacteria ($2.0 \times 10^2 – 7 \times 10^5$ CFU/mL) and fungal counts ($1.1 \times 10^2 – 7.05 \times 10^3$ CFU/mL); *E. coli* was not detected. Identified as *Pediococcus pentosaceus* MD02 and *Pediococcus acidilacti* MD01, these LAB strains exhibited sensitivity at pH 1.5 for a short exposure time of 3 hours and tolerant to moderate acidity of pH 3 for a longer exposure time of 36 hours. The LAB strains further showed 40% – 90% survival rate in a low (0.3%) and high (0.9%) bile salt concentrations after exposure for short (24 hours) and longer (48 hours) periods. *P. pentosaceus* MD02 exhibited a higher antioxidant capacity of 38.85%. The strains showed resistance to streptomycin and penicillin. Mageu pH decreased from 4.2 to 3.2, and total titratable acidity increased from 0.16% to 0.27%. Antioxidant activity was lower than L-ascorbic acid. Proximate analysis revealed increased protein (42 – 58 µg/mL), crude fat (0.54 – 0.96 g/mL), moisture (85% – 99%), and ash (9.8% – 13%). Minerals (calcium, potassium, magnesium, sodium, phosphorus) ranged from 1.2 – 9.6 mg/mL, and sugars (glucose 2 – 47 mg/mL, sucrose 4 – 96 mg/mL) were detected. Sensory evaluation varied, with mageu 5:5 preferred. Millet-based mageu, fermented with LAB, demonstrated probiotic properties, nutritional value, and favourable sensory attributes and the natural bitter taste of millet was neutralised. Millet emerges as a highly nutritional alternative to staple cereals. This study highlights the potential of millet as a mainstream, health-promoting food source, addressing the demand for diverse and nutritious alternatives to traditional cereals like maize, sorghum, and wheat.

CHAPTER 1. INTRODUCTION

1.1. Background of the study

Food insecurity and good nutrition is a global challenge, and the African continent is largely affected, with large population size and high disease incidences being the significant contributors (Gabaza *et al*., 2019). Maize, sorghum and wheat are the main food crops in the African communities. Developing countries experience poverty and malnutrition due to large population size (Gabaza *et al*., 2019) and the poor nutritive quality of the available food products.

It is important to develop and improve the nutritive value of these food products in order to sustain and nourish the health of the African population. About 80% of the South African population relies on maize as a staple food and the increase in population, poor soil fertility, water resource and limited land are presently distressing maize production capacity (Mutsamba *et al*., 2020; Zhao *et al*., 2020). Millet is drought resistant, can withstand high temperatures and has a better nutritional profile than maize and sorghum (Saldivar, 2003; Taylor, 2016). It is not commonly consumed like maize and there is a dearth of knowledge on the health and safety benefits of fermented millet food products. This is with consideration that the use of fermentation in food production improves the flavour and nutritive value of food products and consequently produces food products with longer shelf life (Panda *et al.,* 2014). Moreover, fermented grains not only provide essential nutrients for humans but also contain antimicrobial properties and probiotics (Adisa *et al.*, 2020). As a result, millet has a great potential for inclusion into the mainstream food basket to curb the effect of food insecurity, notwithstanding the need to improve its processing to address its natural bitter taste.

Mageu is a fermented food product commonly produced from maize flour, or leftover maize porridge as well as from a combination of sorghum and maize in African communities. The studies of Katangole (2008) and Simatende (2015) described mageu as a fermented non-alcoholic maize-based drink that is used as weaning food for babies and consumed at different ceremonies (Idowu *et al.,* 2016). Mageu is produced through spontaneous fermentation (Eswatini, 2019). The safety of mageu

was reported for the bacteriostatic and bactericidal properties against enteric pathogens (Ojokoh *et al.,* 2015). Lactic acid bacteria (LAB) and yeasts (mostly non-fermenting yeasts) were found to be the predominant microorganisms in mageu, amongst other microorganisms such as enteric bacteria and moulds (Katangole, 2008). Lactic acid bacteria are also included as probiotics (Somashekaraiah *et al*., 2019).

The study explored an introduction of millet as an alternative cereal meal to maize in the making of traditional African food products. There is an interest in the fermentation of cereals such as sorghum, maize and millet for the production of food with better nutritional profiles (Adebo, 2020; Madoroba *et al*., 2011). Millet is more nutritious than other cereals since it contains significant quantities of essential amino acids and higher amounts of macronutrients such as starch, protein and lipids, vitamins and minerals (Amadou *et al*., 2011). In addition, millet has good health properties such as anti-inflammatory, anti-hypertensive, anti-mutagenic, anti-oestrogenic and anti-carcinogenic characteristics, and the presence of antioxidants compounds make millet a good candidate for development of nutraceutical products (Duodu and Awika, 2019).

1.2. Aim

The aim of the study was to investigate the selected microbiological, nutritional and health properties of mageu produced from millet and maize flours and to evaluate the probiotic potential of the autochthonous Lactic acid bacteria.

1.3. Objectives

The aim of the study was achieved through the following objectives:

i. Isolation, characterisation, and identification of fermentation-contributing microbiota with specific focus on lactic acid bacteria.
ii. Investigation of the probiotic property of the lactic acid bacteria (acid tolerance, bile salts tolerance).
iii. Investigation of the safety properties of the lactic acid bacteria (antibiotic susceptibility test, antioxidant activity).

iv. Chemical characterisation of the beverage (total titratable acidity, antioxidant activity).
v. Determination of nutritional content of the beverage (total sugar, protein content, fat content, moisture content, ash, and mineral content).
vi. Sensory profiling of the fermented brew.

1.4. Research hypothesis

The fermented milled-based beverage (mageu) has a unique nutritional profile and health properties that are beneficial and can curb the challenges of food insecurity.

CHAPTER 2. LITERATURE REVIEW

2.1. What is millet?

Millet **(*Panicum miliaceum*)** is one of the important cereals for food production, especially for the African continent. However, it has not received wide usage to date as a mainstream cereal, similar to maize, wheat and sorghum. Millet is a small, seeded grain belonging to the *Poaceae* plant family and it is acknowledged as the *Gramineae* (Pradeep and Sreerama, 2018). It is easily cultivated, can resist drought, and survive under extreme weather conditions such as high temperatures, high salinity and low rainfall (Dias-Martins *et al*., 2018). Millet cereal grains are in abundance with noble nutritional profile and the ability to curb many health-related issues such as hypertension, cardiovascular diseases, thyroid, cholesterol, obesity, oxidative stress, and celiac diabetes (Nithiyanantham *et al*., 2019). It is often used without processing following harvesting and cleaning (Bhat *et al*., 2019). This could be attributed to the retention of most of the nutrients. Millet is widely grown in Africa, India, China, Europe (mainly Russia) and Asia and humans have been consuming millet for about 7000 years to date (Vanishree *et al*., 2018).

2.2. Types of millet

There are different varieties of millet. These are classified into major and minor (also called small millet) millets. The most cultivated and popular major millets are pearl millet (*Pennisetum glaucum*), foxtail millet (*Setaria italica*), proso/white millet (*Panicum miliaceum*) and finger millet (*Eleusine coracana*) (Bhat *et al*., 2019). Small millets include barnyard millet (*Echinochioa* spp.), kodo millet (*Paspalum scrobiculatum*), little millet (*Panicum sumatrense*), guinea millet (*Brachiaria deflexa*) and brown top millet (*Urochloa deflexa*) (Muthamilarasan and Prasad, 2021). Figure 2.1 shows different types of millets with their typical appearances in terms of colour and size.

Figure 2.1: Different types of major and small millets (https://milletadvisor.com/millets-types-of-millets).

The different types of millet differ by their nutritional composition and nutrient bioavailability which is informed by their digestibility. Millet is generally known as leotša in Sepedi, nyalothi in Sesotho and umyaothi in isiZulu.

- Pearl millet has total production of 76% (Taylor, 2016) with the largest production in India (Adebo, 2020). In comparison to other types of millets, pearl millet has high energy and niacin content, and it is rich in unsaturated fats and calcium (Dhedhi *et al*., 2016). Furthermore, it has protein content of 12 – 16%, about 11.5% of dietary fibre and 4 – 6% lipid content. These are sufficient for good health in the diet of humans.

- Finger millet (also known as ragi) is preferentially used in weaning foods for children. Taylor (2016) reported a total production of 19% for finger millet. This variety is rich in mineral content and has high antioxidant activity. Finger millet is regarded as a rich source of calcium (300 – 350 mg/100 g) and has protein content of 6 – 8% while the fat levels are considerably low (Antony, 2018).

- White/ proso millet has unique nutritional content that contributes towards the reduction of cardiovascular diseases due to the ability to decrease the triglycerides and C- reactive protein thereby prohibiting heart diseases. There

is not much reported about this variety, however, it is considered as the cheapest source of manganese and has noticeable amounts of proteins (12.5%) and calcium (Johnson *et al*., 2019).

- Foxtail millet is regarded as the sweet millet when compared to the other types due to its nutty sweet flavour as well as its unique digestibility (Nithiyanantham *et al*., 2019). Foxtail millet is free of allergens, has high protein content, although not quantified, it is assumed to be double that of other cereals such as rice (Nithiyanantham *et al*., 2019). Millet is reported to have high carbohydrate content, as well as minerals such as copper and iron (Lightfoot *et al*., 2016).

2.3. Millet as a food cereal

Millet is consumed by up to 90% of the population in countries such as India and Russia (Adebiyi *et al*., 2018). However, this is not the case in Africa, especially South Africa wherein maize is still the dominant cereal, followed by sorghum and wheat, all with products in the mainstream market. As such, maize, sorghum and millet are generally regarded as the most significant staple food sources in developed and developing countries (Gabaza *et al*., 2019). Their cultivation is for human consumption including weaning of children.

Most recently, maize depletion is an increasing concern globally because of escalating population. However, with maize being considered as the staple food crop in Africa, its depletion contributes to food insecurity and consequently poverty and hunger. Currently, production of maize is affected by unfavourable weather conditions such as low rainfall, high temperatures, limited land and low soil fertile characteristics (Adebiyi *et al*., 2018).

Millet has great advantage and good potential for mainstream food source due to its exceptional nutritional composition for the promotion of good health. Furthermore, it is easy to maintain and adaptable to unfavourable weather conditions (Adebiyi *et al*., 2018) in contrast to maize. Hence, it can be used as an alternative to the depleted maize crop. Millet is generally used and consumed with little processing. Unfortunately, it imparts a bitter taste, and this makes it to be less liked and less not

widely adopted in spite of its good nutritive value. Various food processing methods can mitigate the bitterness and improve its wide usage.

2.4. The use of fermentation in food production

Fermentation is one of the food processing techniques commonly used in food production. It is a simple cost-effective way of preserving food and producing unique food products. The process of fermentation depends on the actions of various microorganisms such as yeasts and lactic acid bacteria (LAB) for production of desirable metabolites (Adebiyi *et al*., 2018). Fermentation processes can be varied by changing processing conditions (Moodley, 2015).

Fermentation can either follow a natural (Spontaneous) or a controlled process. With controlled fermentation, the process is monitored and performed under optimal conditions with addition of starter cultures whereas the natural fermentation process is not monitored (Adisa *et al*., 2020). Most home-based fermented products are produced through spontaneous fermentation, especially in the African continent (Habegger *et al*., 2018). Starter culture-based/ inoculum-based fermentation process has an advantage of producing consistent products from one batch to the other due to the controlled conditions of processing whereas spontaneous fermentation may produce products differing with sensory properties, nutrients, palatability and shelf life due to the process being uncontrolled (Moodley, 2015).

There are various fermented beverages, presumed to be about 5000 (Grumezescu and Holban, 2019) and traditionally fermented foods, which are consumed worldwide especially in African communities (Adebo, 2020). These include products such as bread, thin or thick porridges, non-alcoholic and alcoholic drinks. The porridge and non-alcoholic beverages serve as nutritious food for children, as well as weaning food for infants (Gabaza *et al*., 2019).

Generally, fermented foods are prepared from a variety of sugar containing grains (maize, millet, sorghum, wheat, rice), fruits and honey (Grumezescu and Holban, 2019). Fermented food and beverages differ by fermentation period, composition of starting material as well as microbes used as starter cultures or microbes that drive

spontaneous fermentation. Some common fermented products include mageu, bushera and munkoya, which are categorised as non-alcoholic drinks (Baschali *et al*., 2017: Grumezescu and Holban, 2019) whereas ting, koko and ogi are classified as thick/thin porridges. These fermented food products are good for lactose intolerance individuals and vegetarians.

2.5. Some common fermented food products

- Ting is produced mainly in South Africa, Lesotho and Botswana (Agyei *et al*., 2020). It is a fermented porridge which is liked for its sour taste, and it is produced by spontaneous fermentation using mainly sorghum flour or maize. The production of ting is carried out mainly in households with similar methods of productions throughout the country. Its preparations involve soaking cereals (either millet, sorghum or maize) with warm water followed by incubation for 3 – 4 days at ambient temperatures (Agyei *et al*., 2020). After that, it is then cooked and served either as a soft or thick porridge which can be eaten during breakfast, lunch or supper. Sometimes minor modifications to the production of ting are performed due to personal preferences. It is consumed by infants, children and adults (Lei *et al*., 2019). The nutritional content of ting is high, it is rich in antioxidants and good for individuals with gluten tolerance (Almaski *et al*., 2017).

- Munkoya is popular in Democratic Republic of Congo (DRC) and Zambia (Mashau *et al*., 2021). The making of munkoya involves the use of maize meal, maize grit and roots extracts of *Rhynchosia insignis* (or *Rhynchosia heterophylla*) of which these ingredients are mixed together followed by boiling the mixture for 8 hours and fermented at room temperature (25°C – 30°C) for 24 – 48 hours (Mashau *et al*., 2021). Some people prefer to consume munkoya while it is still fermenting whereas others prefer to consume the beverage after fermentation as a thick or thin porridge. It is preferably used as a weaning drink for infants and consumed as a breakfast or lunch by adults, and mostly regarded as an energising drink (Mashau *et al*., 2021). The flavonoid content of munkoya is high and consumption of this product will be beneficial towards

fighting of diseases especially those causing oxidative stress such as cancer, cardiovascular diseases and obesity (Moonga *et al*., 2020).

- Millet porridge called koko is produced and consumed by the Northern Ghana people (Newlove *et al*., 2022). They use spices such as ginger, cloves and black/chilli pepper before natural fermentation takes place (Danson *et al*., 2019) and it is cooked to different consistencies based on personal preferences (Atter *et al*., 2021; Newlove *et al*., 2022). The spices used to produce koko give it a unique flavour. Koko can be consumed by children and adults as an afternoon snack. Importantly, koko has abilities of lowering diabetes because of high magnesium content that controls glucose receptors in the body (Gibson *et al*., 2022) and can also lower cholesterol due to its fibre content (Gibson *et al*., 2022).

- Bushera, also called obushera, is a beverage produced from a combination of sorghum and millet which is consumed mainly in Uganda. Its preparations involve the mixing of hot water with sorghum and millet. It is consumed by both children and adults (Mbina *et al*., 2019) and as a refreshing drink in social gatherings (Taylor and Duodu, 2019). It is reported that bushera calms the nervous system since it is alkaline, and it is as well regarded as a good source of gluten free fibre (Baniwal *et al*., 2021).

- Akamu (also known as Ogi) is one of the fermented porridges consumed in countries such as Nigeria and Benin (Menezes *et al*., 2018). Akamu preparation requires lots of water. It is prepared from sorghum, millet or maize which are soaked in water to increase the moisture content (Obiegbuna *et al*., 2019) thereby softening the grain for ease of milling (Iyare *et al*., 2020). The filtered mixture is then cooked into a thick porridge after three days of natural fermentation has taken place (Iyare *et al*., 2020). It is sour in taste and most people prefer adding sugar to the porridge prior consumption (Omemu *et al*., 2018). It is consumed any time of the day and mostly recommended for children because of its easy digestion. Ogi is good for the kidney, can lower cholesterol as well as blood pressure regulation (Olaniran and Abiose, 2019).

- Mageu (Figure 2.2) is a traditional fermented drink that is part of the diet of African people (Mashau *et al*., 2020) and it is very common in Botswana, South Africa and Lesotho (Alavi *et al*., 2019; Olusanya *et al*., 2020). Mageu production is popular in the above-mentioned countries because it is associated with values such as diet therapy and religion. In other nations such as Mozambique, more than 70% of the population consider mageu as the staple food. The fermentation process of mageu is a short cheap process. Mageu is produced from natural fermentation of maize meal, sorghum or left-over maize porridge at temperatures between 20 and 30 °C. Mageu has a pH range of 2.4 – 3.5 with little or no amount of alcohol as well as acidity ranging from 0.4 – 0.5%. These amounts of acids and pH normally develops after 2 – 4 days of the uncontrolled fermentation at ambient temperatures (Eswatini, 2019). In South Africa, the production of mageu is seasonal since most people prefer the beverage in summer and not in winter. The drink is consumed by all the age groups, namely, children, infants and adults. The estimated consumption for adults is around 12 – 14 L (Mashau *et al*., 2020).

Figure 2.2: Buckets containing mageu (Misihairabgwi and Cheikhyoussef, 2017).

2.6. Importance of fermented foods in food security

Many people in different nations rely on traditionally fermented foods and beverages for food preservation and stocking up food reserves and thereby lessening the scourge of food insecurity (Adebo and Medina-Meza, 2020; Fusco *et al*., 2017). Generally, food security is defined as the economic and physical access to enough nutritive and protected food for a healthy lifestyle (Saint Ville *et al*., 2019). About 800 million people in developing nations are affected by hunger and poverty (Onyango, 2014). The food

crisis is elevated by increased population sizes and prices of food. Therefore, fermentation of differing grains, more especially millets can be used as a comprehensive approach towards poverty and hunger alleviation and thereby promoting preservation of food with good probiotic qualities and increased nutrition (Abe, 2019).

2.7. Microorganisms and food fermentation

Microorganisms are defined as the organisms that cannot be seen by the naked eye and these include bacteria, yeasts, viruses, and moulds. These organisms break down complex compounds, synthesising complex vitamins as well as other growth factors (Sharma *et al*., 2020). The bacterial group called Lactic acid bacteria (LAB) are important in food fermentation, followed by yeasts. Although there are moulds and other species of fungi that also contribute to food fermentation. LAB are known to produce lactic acid during fermentation as well as their abilities to inhibit harmful undesirable bacteria. Lactic acid bacteria such as species of *Pediococcus*, *Lactobacillus*, *Enterococcus*, *Leuconostoc*, *Weissella* as well as *Lactococcus* are associated with the fermented foods, both alcoholic and non-alcoholic beverages (Oh and Jung, 2015; Tamang *et al*., 2016) On the other hand, yeasts are known for their beneficial components of aroma and production of alcohol in the fermented food product (Tamang *et al*., 2016). Yeasts species such as *Saccharomyces*, *Candida*, *Pichia*, *Hansenula* have been isolated from fermented food and beverages (Tamang *et al*., 2016). These fermenting bacteria and yeast species provide both the physical and chemical changes to the fermented products (Sharma *et al*., 2020). The fermenting microorganisms increase the availability of nutrients, enhance probiotic attributes, degrade anti-nutritive and toxic components and improve the sensory character of the food product (Rezac *et al*., 2018). They also contribute to some health promoting effects such as antimicrobial and antioxidants properties (Tamang *et al*., 2016). Apart from the above-mentioned factors, they also enhance the shelf life of the food products. Some of these species are preferably used as starter cultures for production of various fermented food products due to their good benefits (Sharma *et al*., 2020).

The discovery of microorganisms associated with food fermentation made it possible for understanding and managing of these organisms (Tamang *et al*., 2016), hence it is currently possible to prevent undesirable factors of organisms in food production (Bonatsou *et al*., 2017). The shortcoming of spontaneous fermentation is the possible presence of undesirable microorganisms that emanate from the raw materials, and these may flourish and produce toxins, mycotoxins, cause spoilage of the product during fermentation or storage as well as causing the off taste in food by producing undesirable metabolites (Adisa *et al*., 2020). These microorganisms are regarded as undesirable since they are not beneficial but detrimental to the health of human beings. To the contrary, the organisms which have good probiotic potential are desirable and they contain live bacteria that when consumed in required amounts can benefit the health of an individual since they have abilities to proliferate and colonise the gut, combat gastric juices as well as resisting exposure to bile salts (Bonatsou *et al*., 2017) and more importantly aid the digestion and release of nutrients from consumed food products.

2.8. Chemical characteristics of fermented food products

Raw materials or ingredients that are used in fermentation of various food products play a major role towards characterising the chemical properties of the products (Khan *et al*., 2022). Cereal grains such as sorghum, maize, wheat, fruits and wheat contain higher amounts of antioxidants and phytochemicals such as flavonoids, polyphenols, tannins and phenolics in the bran and germ of the whole grain kernels (Figure 2.3) (Khan *et al*., 2022). The above-mentioned biochemical compounds provide human beings with some of the health properties such as protection against cancers and cardiovascular diseases, maintenance of blood sugar levels and digestive system. Although different types of organic acids may be added or produced through fermentation and fortification, other types of phytochemicals in the whole grains capable of promoting health are not replaceable. However, it is advisable to use the whole grains instead of the refined grains due to their noble benefits (Khan *et al*., 2022). The difference between the two layers of the grain, whole grain and refined grain are shown below in figure 3.

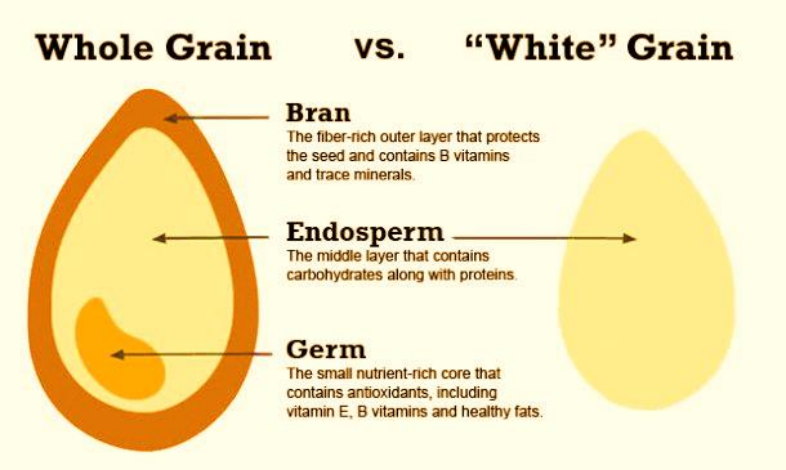

Figure 2.3: Anatomy of the whole grain and the refined grain along with the benefits of each layer (https://www.google.com/url?sa=i&url=https%3A%2F%2Fcooking%2Fhealthy).

Microorganisms also play a crucial role in the chemical characterisation of fermented food products. In addition, the extracts of microorganisms such as yeasts and LAB are known as good sources of antioxidants (Bonatsou *et al*., 2017). LAB are used most often for fermentation of food and beverages due to their abilities to decrease the pH as well as increasing the total organic acids (Zhao *et al*., 2020). They produce acids such as lactic acids and acetic acids which contributes towards safety of the fermented food by managing the microbial stability as well as inhibiting the growth of spoilage microorganisms (Mannaa *et al*., 2021). These microorganisms are also rich sources of different types of organic acids (such as malic, citric, succinic, folic, oxalic, propionic and iso-butyric acids), alcohols and volatile compounds (Bressani *et al*., 2020). These acids determine the quality of the food product in addition to their preservative properties (Mannaa *et al*., 2021) by contributing to the flavour, smell, colour, palatability, digestibility along with prolonged shelf life (Fusco *et al*., 2017).

2.9. Nutritive value of fermented products

Fermentation is recognised for producing healthy foods (Moodley, 2015) that are more nutritious compared to the unfermented foods (Sharma *et al*., 2020). Fermentation increases the bioavailability of nutrients thereby enhancing the nutrient density. The escalated nutritional content in the fermented foods is caused by the utilisation of raw materials that are not refined as well as the fermenting microorganisms existing in them (Narkthewan, 2020). Such microorganisms will break or decompose the cellular

walls and indigestible coatings during fermentation thereby increasing the amount of vital nutrients. Lactic acid bacteria, yeasts and some of the moulds are responsible for fermentation (Mashau *et al*., 2020). The processes of lactic acid fermentation by LAB strains promote solubility of proteins along with availability of some micronutrients and macronutrients (Moodley, 2015). Fermentation improves the composition of macronutrients because of the amylolactic activity of LAB that hydrolyses starch thus enhancing digestibility and energy content of fermented foods. Generally, the fermentation process activates hydrolytic enzymes that degrade polymers into simple nutrients, which thus become readily available for use during consumption of the fermented food products (Narkthewan, 2020).

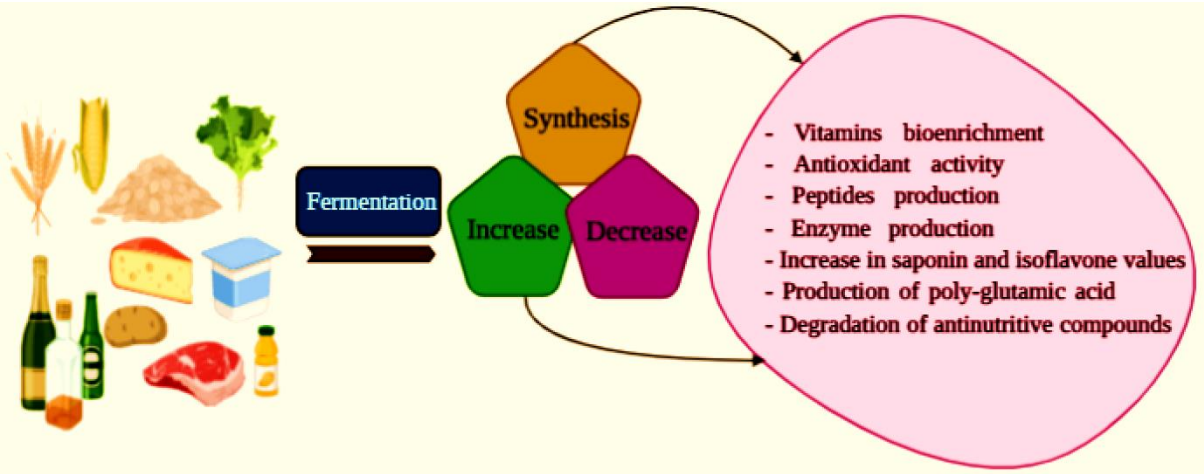

Figure 2.4: Nutritional increment of fermented foods (Source: Sharma *et al*., 2020).

Fermentation improves nutritive value of food products by elevating the levels of nutrients such as minerals, bioactive compounds, dietary fiber, vitamins (with vitamin B complex dominating), proteins, carbohydrates, fats and moisture content. Moreover, nutritional enrichment such as increased extraction of minerals more especially phosphorus, potassium, magnesium, calcium, zinc and iron, trace elements and proteins from grains during fermentation has been reported (Moodley, 2015). These essential nutrients provide about 60 – 70% of energy and immune boosting capacity to humans thereby promoting good health (Baniwal *et al*., 2021; Narkthewan, 2020).

2.10. Health properties of fermented food products

The essential element for a productive and healthy lifestyle is recognised as access to adequate nutrition. To date, health challenges and poor nutrition continue to be a crucial global challenge. Some of the health challenges such as growth, immunity, development and general wellbeing result from lack of essential nutrients such as proteins, vitamins, minerals and antioxidants (Olusanya *et al*., 2020). Traditionally fermented beverages and foods are known for their health promoting properties (Baschali *et al*., 2017). They are regarded as functional foods due to being rich in beneficial nutrients, probiotics and other properties for a healthy gut.

Lactic acid produced in fermented foods such as mageu, ogi, ting, koko and other types of fermented foods commonly prevents fat accumulation which consequently lowers cholesterol, obesity as well as type 2 diabetes (Narkthewan, 2020). Some of these foods have anti-aging characteristics due to production of melatonin that is known to regulate the body clock (Tamang *et al*., 2016). The presence of probiotics and bioactive compounds such as phenolics, flavonoids (mainly quercetin, vitamins E and C, and mineral selenium) in fermented whole grain foods lowers the risk of cardiovascular diseases by reducing incidences of heart attacks along with mortality rates (Tamang *et al*., 2016). Vitamins K and C assist in the activation of natural antioxidants enzymes which aid in immune modulation while the dietary fiber can supress growth of cancer cells as well as detoxifying heavy metals in the liver, kidneys and small intestines (Tamang *et al*., 2016). Moreover, consumption of fermented food rich in fiber content may control diabetes because of their ability to increase insulin resistivity. Minerals such as phosphorus, calcium, potassium (Mathipa, 2022) as well as vitamin K2 stimulates formation of healthy bones which may help in preventing osteoporosis especially in older man and women (Odunmbaku *et al*., 2018). Proteins present in fermented foods such as bushera, millet porridge koko, motogo and others also function to promote strong bones, teeth and muscle health (Geissler and Powers, 2017). The LAB, on their own are good probiotics that can therapeutically curb disorders of the stomach by controlling inflammatory bowel diseases such as diarrhoea and constipation as well as symptoms associated with lactose intolerance (Grumezescu and Holban, 2019). Some of the health advantage includes improving

digestive health, blood circulation, immune system and cellular metabolism (Baniwal *et al*., 2021; Narkthewan, 2020).

2.11. Safety characteristics of fermented food products

Fermented foods are regarded as safe to consume due to possessing factors that inhibit the survival and growth of unwanted microorganisms (Baschali *et al*., 2017; (Somashekaraiah *et al*., 2019). Spoilage microorganisms do not survive in acidic environments, which result from the bacterial activities during the fermentation process. Inadvertently, the low pH plays a critical role on enzymes that break down phytate to release minerals such as zinc, iron and calcium resulting in unfavourable conditions for undesirable microorganisms (Mashau *et al*., 2021). Fermented foods are rich in probiotics; and these bacteria prevent pathogenic microorganisms from thriving in the human gut by decreasing the pH inside the gastrointestinal tract. Thus, reducing the occurrence of intestinal infections (Maakelo *et al*., 2021). Sharma *et al*. (2020) recently demonstrated the downregulation of *Helicobacter pylori* infections in humans by the effect of probiotics. Fermented foods like tongwa, ogi and mageu are indicated as having antimicrobial properties such as niacin, pediocin and bacteriocin, thereby protecting against food-borne pathogens and inhibition of dangerous strains of *Escherichia coli*, *Enterococcus faecalis*, *Salmonella typhimurium* and *Listeria monocytogenes* (Adisa *et al*., 2020).

The activity of LAB further adds to safety by degrading the anti-nutritional factors in the fermented foods making them safe for consumption (Adisa *et al*., 2020). This also contributes towards extension of shelf life by the controlled microbiota along with antimicrobial compounds produced by LAB strains such as hydrogen peroxide, antifungal compounds (propionate, hydroxyphenyl-lactate) and low molecular weight metabolites (diacetyl, fatty acids) (Licandro *et al*., 2020). Most LAB species produce hydrocyanic acid that is known to make foods products non-toxic leading to safer consumption by humans (Sharma *et al*., 2020).

2.12. Sensorial factors in fermented food products

Fermentation process enhances the sensory attributes such as flavour, taste, texture and aroma of food products. These characteristics influence consumers' acceptability

of the fermented food products. The sensorial factors vary depending on the process of fermentation, temperature, incubation period and storage conditions (Fushiki and Nakano, 2019). Microbial growth plays a major role towards sensory attributes hence the LAB present in fermented foods cause the smell and taste to be slightly sour and bitter due to the production of organic acids. However, not only LAB cause sourness and bitterness, the interaction of proteins and bioactive compounds such as tannins contributes towards sourly taste as well, especially in whole grain and fruit fermented food products (Wikandari *et al*., 2021). Adding to taste, the bitterness is also influenced by isoflavone glucosides which are hydrolysed as fermentation progresses to isoflavone aglycones. On the other hand, the saponin coating in millets crops also contributes towards the sourness of millet fermented food products (Agyei *et al*., 2020).

Organic acids, the activation of certain enzymes along with microbial metabolites all cause changes in texture of the fermented food products. Fermented food products such as ting, ogi, millet porridge and koko have soft texture due to the mineral composition and high moisture content (Zanra *et al*., 2022). The fat content plays a significant role towards softening of the fermented food products. On the other hand, the colour of most fermented food products is influenced by factors such as high moisture content, fat content, dietary fiber, carbohydrates along with raw materials used (Mathipa, 2022). The combination of flavour, texture, taste, smell and colour bring about balance and harmony of fermented foods (Fushiki and Nakano, 2019).

2.13. An ideal fermented food product

Fermented food products continue to be explored by different people worldwide due to food insecurity (Zanra *et al*., 2022). To date, there are many fermented food products produced and many are being developed due to the many benefits and simple fermentation processes. An ideal fermented food product will have essential nutrients such as high amounts of proteins, mineral content, vitamins and dietary fiber (Mashau *et al*., 2021). Good nutrition benefits the population in fighting against malnutrition and other health related issues. The accumulation of various microorganisms, acids, enzymes and antioxidants provide good quality products which are safe for human consumption (Fushiki and Nakano, 2019). Fermented foods

should have prolonged shelf life, be palatable and be easily digestible. When such a product is produced from easily accessible material and is cost effective, then great strides would have been made in combating the ill effects of malnutrition and food insecurity.

2.14. Purpose of the study

Globally, poverty, hunger and malnutrition are identified as the major challenges with high prevalence in developing countries. Crops such as maize are getting depleted because of adverse factors such as poor soil fertility, water resources (Mutsamba *et al*., 2020) as well as limited land space, which together pose a serious challenge in maize production (Zhao *et al*., 2020). The depletion of maize as the primary staple cereal crop contributes towards food insecurity (Walch *et al*., 2018). Apart from that, maize lacks some of the essential nutrients such as proteins, vitamin A and E as well as zinc that humans need (Olusanya *et al*., 2020) to build a good immune system. Interestingly, grains such as millet are underrated, even though it possesses unique beneficial health and nutritional properties (Duodu and Awika, 2019). Its less popularity could be attributed to its bitter taste which is due to its saponin coating. Processing of millet through fermentation was applied with intent to improve palatability of millet. Vinoth and Ravindhran (2017) previously suggested fermentation of millet to enhance its usage and acceptability. This study explored an introduction of millet as a highly nutritive alternative cereal to maize in the making of mageu. Mageu was produced following the natural fermentation process that relies on the natural flora present in the grains. The intent was to produce a traditional millet-based fermented beverage that is rich in probiotics, has improved nutrition and novel sensory attributes as compared to the traditional mageu made from maize. Kumar *et al*. (2021) commented that the introduction of millet in the mainstream food basket can potentially reduce the demand on maize, thus addressing food insecurity.

CHAPTER 3. DETERMINATION OF THE FERMENTATION /PARAMETERS FOR MILLET BASED FERMENTED MAGEU BEVERAGE PREPARATIONS AND TO INVESTIGATE THE PROCESS OF FERMENTATION BEFORE FULL-SCALE STUDY TAKES EFFECT: A PILOT STUDY

3.1. Abstract

Millet (*Panicum miliaceum*) is one of the most important cereals in Africa. Millet has unique nutritional profile which gives it an advantage for health promotion over other cereals. Consequently, retention of these nutrients during processing of millet into food products can help curb the ill effects of malnutrition and food insecurity. This study aimed to determine the selected microbiological, chemical properties and sensory attributes and shelf life of varying proportions of millet-based mageu (mageu 9:1, mageu 8:2 and mageu 5:5). De Man Rogosa and Sharpe (MRS) agar medium was used for isolating lactic acid bacteria (LAB) whereas yeast extract peptone dextrose (YPD) agar was used for the yeasts. Faecal and total coliforms were determined using membrane filter technique. The procedures used for differentiating the LAB were the colony and cellular morphology, biochemical testing comprising of catalase, motility, sugar fermentation, citrate utilisation, growth test at different salt concentrations and temperatures. The pH was measured using pH meter and total titratable acidity (TTA) using titrimetric method whereas specific gravity was used to get degrees Brix (°Bx). Three candidates participated in determining the sensory attributes of mageu. Shelf life was evaluated based on chemical and sensory attributes following storage at 4 °C for 5 weeks. LAB (4.3×10^5 – 20.0×10^5 CFU/mL) were increasing while the yeasts (10.3×10^5 – 4.3×10^5 CFU/mL) were decreasing in all the ratios of millet-based mageu while total and faecal coliforms were not detected. Two isolates with Gram-positive coccus and coccobacillus shaped cells were observed along with similar catalase, motility, and citrate utilisation negative tests. The pH declined from 6.01 − 3.05 as fermentation progressed with inverse proportional increase in total titratable acidity from 0.03 − 0.4%. The sugar content was within 2.4 – 4.6 °Bx. The organoleptic properties were different in all the ratios. The pH ranged from 3.19 −3.01 whereas TTA ranged from 0.2 – 0.5% during storage and the mageu retained their organoleptic

attributes. The increasing TTA was influenced by the production of lactic acids by LAB with low pH. The tasting panel appreciated mageu 9:1 and mageu 5:5 except for mageu 8:2. Production of mageu using millet flour improved the LAB count and the palatability of mageu.

3.2. Introduction

Mageu is a traditional non-alcoholic fermented beverage produced mainly in South Africa, Botswana as well as Zimbabwe. The preparation of mageu involves the use of maize meal, sorghum, and leftover maize porridge through a spontaneous fermentation process by different microorganisms (Phiri *et al*., 2020). This beverage is consumed by people as a refreshing drink at different cultural ceremonies and it is also used as weaning food for babies.

During mageu production, factors such as the unique taste, palatability, quality, microbial stability, chemicals, nutrition as well as acceptability of the product are guided by the methods of processing (length of fermentation and storage conditions) and the types of grains used (Adebo and Medina-Meza, 2020; Phiri *et al*., 2020). The microbial population isolated from most mageu products are the lactic acid bacteria (LAB) which are classified as the good bacteria for the gut and important in addressing many health-related issues (Olusanya *et al*., 2020). The balance between the chemical composition of mageu such as the lactic acid produced by LAB and pH plays a major role towards the sensorial attributes of the fermented product and further provides the safety of the product by inhibiting growth of unwanted microorganisms (Mashau *et al*., 2020).

This study focused on the use of millet, especially pearl millet flour as an alternative to sorghum and maize meal for production of differing proportions of mageu. Millet is underutilised in Africa mainly due to its bitter taste, although highly nutritive and resistant towards unfavourable weather conditions as compared to maize, sorghum, and wheat (Amadou *et al*., 2013; Taylor, 2016). The use of millet will reduce the reliance on maize meal which is becoming insufficient due to low rainfall. Consequently, its contribution to curbing food insecurity cannot be overstated.

The chapter highlights the fermentation parameters and the factors such as microbiological properties, chemical composition, and sensorial attributes of mageu produced using pearl millet flour.

3.3. Materials and methods

3.3.1. Source of study materials

Maize meal and pearl millet flour were purchased from a local supermarket in Mankweng near the University of Limpopo.

3.3.2. Preparations of millet-based mageu and sample collection

3.3.2.1. Preparations of mageu

The following millet: maize proportions were chosen with a total volume of 700 mL. A modified method of Fadahunsi and Soremekun (2017) was followed, which includes the following:

i. (80 + 10): 10 (millet: maize) (denoted as 9:1)
ii. (60 + 20): 20 (millet: maize) (denoted as 8:2)
iii. (25 + 50): 25 (millet: maize) (denoted as 5:5)

For the first proportion [(80 + 10): 10) (millet: maize)], 80 g of millet flour and 10 g maize meal were mixed in a bowl with 100 mL of potable tap water and mixed thoroughly into a paste. Four hundred millilitres of water were added into a pot and heated to a bubble boiling point. The paste was added slowly with continuous stirring and cooked for 25 minutes. Heating was reduced to about 50 °C and allowed to simmer for 5 minutes. The soft cooking porridge was mixed thoroughly every 5 minutes to homogeneity. After cooking, the soft porridge was poured into a bucket and allowed to cool down to ambient temperature. Another 10 g of millet flour (inoculum) and 200 mL water were added and mixed thoroughly to achieve homogeneity. The gruel was then allowed to ferment naturally at 25 °C in a closed bucket for four days. Thereafter, the bucket containing the mageu was stored at 4 °C in the refrigerator.

The same procedure was followed for the two other proportions using the amounts of millet and maize as indicated in the ratio.

The millet-based mageu 8:2 had 60 g of millet flour mixed with 20 g of maize meal with 20 g of millet flour used as inoculum. The millet-based mageu 5:5 composed of 25 g of millet flour mixed with 50 g of maize meal with 25 g of millet flour used as inoculum.

3.3.2.2. Sample collection and stock cultures preparation

Fifty millilitres (50 mL) of samples from each millet-based proportion were collected every 24 hours for 4 days using 50 mL sterile centrifuge tubes following thorough mixing of the fermenting gruel in the bucket. Thirty millilitres of the sample were centrifuged at 10000 rpm for 5 minutes. For microbiological analysis, the supernatant of the mageu samples was used to prepare stock cultures in 50% glycerol. Two millilitres of mageu supernatant were transferred into 2 mL microcentrifuge tubes and further centrifuged at 5000 rpm for 10 minutes. The pellet was suspended in 1 mL of 0.85% saline solution and 1 mL 50% glycerol and stored at -20 °C. The supernatant was stored at 4 °C until chemical analysis was performed. All preparations were performed in triplicates.

3.3.3. Microbiological evaluation of mageu

3.3.3.1. Isolation and enumeration of bacteria and yeast

Microbiological evaluation of mageu samples was carried out using a culture dependent method. Serial dilution of the samples was carried out from 10^{-1} to 10^{-6} dilutions. De Man Rogosa and Sharpe (MRS, Sigma-Aldrich) agar medium was used for isolating lactic acid bacteria (LAB) using 100 µL of all the diluted samples according to Idowu *et al*. (2016). The isolation of yeasts was done using yeast extract peptone dextrose (YPD, Sigma-Aldrich) agar plates. One hundred microliters of the sample were spread plated on agar media (MRS and YPD) using 5 mm sterilised beads. The MRS agar plates were incubated anaerobically at 37 °C for 48 hours whereas the YPD agar plates were incubated aerobically at 30 °C for 48 hours. Plates with colony counts in the range of 30 to 300 were enumerated manually using the viable count method

and recorded as colony-forming units per millilitre (CFU/mL) following the equation below:

$$CFU/mL = \frac{number\ of\ colonies \times dilution\ factor}{volume\ plated}$$

Testing for faecal and total coliforms were done using m-Faecal coliforms (mFC, Sigma-Aldrich) and McCarthy Delaney Grasso (m-Endo, Sigma-Aldrich) media respectively and prepared according to the manufacturer's instructions. Membrane filter technique was used for the detection and enumeration of both the total and faecal coliforms. One millilitres of mageu stock culture was serially diluted (10^{-1} to 10^{-6}) with 49 mL of sterile 0.85% of saline solution and thereafter, the 49 mL of the mageu sample was filtered aseptically and the filter paper was incubated in lauryl sulphate broth (Sigma-Aldrich) for 2 hours at 35 °C and thereafter transferred to an m-Endo plate (Baird *et al*., 2015; Jorgensen *et al*., 2015; Salfinger and Tortorello, 2015). This was also performed for faecal coliforms using mFC media except for the lauryl sulphate broth incubation. The dilutions from 10^{-1} to 10^{-6} in triplicates were used for plating. The m-endo plates were incubated at 35 °C for 24 hours and the mFC plates at 45 °C for 24 hours. Viable counting of colonies ranging from 30 to 300 was done manually and recorded as CFU/mL.

3.3.3.2. Purification of the isolates

Different colonies on the MRS agar were randomly chosen based on differences in colour, shape, margin, elevation, and size. Gram staining was performed, and cell morphology and staining reaction were recorded.

Purification of LAB was performed by sub-culturing on the same culture medium. The selected LAB isolates were repeatedly sub-cultured on MRS agar and incubated anaerobically at 37 °C for 48 hours until pure cultures were obtained. The pure cultures were stored at 4 °C.

3.3.4. Biochemical characterisation of selected lactic acid bacteria isolates

3.3.4.1. Catalase test

Cultures that are not older than 24 hours were added aseptically into test tubes with 2 mL of hydrogen peroxide using a sterilised inoculating loop. *Pseudomonas aeruginosa* American type culture collection *(*ATCC 9027) was used as the positive control. A positive result was noted when immediate bubbling was observed (Ismail *et al*., 2018).

3.3.4.2. Salt tolerance assay

MRS broth media with either 5%, 6.5% or 13% sodium chloride (NaCl, Sigma-Aldrich) were prepared. The cultures were aseptically inoculated into tubes using a sterilised inoculating loop. The test tubes were closed tightly and incubated for 7 days at 37 °C. The increase in turbidity indicated a positive result (Ismail *et al*., 2018). *Enterococcus faecalis* (ATCC 29212) was used as a positive control.

3.3.4.3. Growth temperature assay

A colony of the LAB isolates were inoculated into tubes containing 10 mL MRS broth. The test tubes were incubated at 25 °C and 37 °C for 7 days. *P. aeruginosa* (ATCC 9027) was used as a positive control. The increase in turbidity indicated a positive result (Ismail *et al*., 2018).

3.3.4.4. Motility test

Sulfur, indole, and motility agar (SIM; Sigma-Aldrich) slants were prepared according to the manufacturer's instructions. A sterilised inoculating needle was used to inoculate the middle of the agar tube. The tubes were incubated for 7 days at 37 °C while observing the growth by checking the diffuse zones showing growth from the inoculation line (Ismail *et al*., 2018). *E. coli* (ATCC 25992) was used as a positive control.

3.3.4.5. Citrate utilisation assay

Simmons citrate (Sigma-Aldrich*)* agar slants were prepared in test tubes according to the instructions of the manufacturer. All the cultures were transferred into the tubes by streaking on the slants. The tubes were incubated aerobically for 7 days at 37 °C to

observe the colour change from green to blue, indicative of the ability to use citrate as an energy source (Ismail *et al*., 2018). *Klebsiella pneumoniae* (ATCC 10102) was used as a positive control.

3.3.4.6. Sugar fermentation assay

One gram (1 g) of lactose, sucrose and glucose were separately added to a solution containing 1.6 g nutrient broth, 1 g peptone, 0.3 g meat extract, 0.5 g NaCl, 100 mL distilled water and 8 mg phenol red (pH indicator) in test tubes. Durham tubes were added to the test tubes for determining the fermentative ability of the cultures. Cultures were used to inoculate the different sugar media and were incubated at 37 °C for 24 hours with *E. coli* (ATCC 25992) as a positive control. The presence of bubbles in the inverted Durham tubes was taken as a positive result for fermentation while the absence of bubbles meant a negative result (Ismail *et al*., 2018).

3.3.5. Chemical characterisation of mageu

3.3.5.1. pH

A 10 mL sample of the supernatant from the centrifuged mageu sample as outlined in section 3.3.2.2 was transferred into a 50 mL glass beaker and the pH was recorded at 25 °C.

3.3.5.2. Determination of total titratable acidity

The same sample used for pH test was used, and 10 mL of distilled water was added to the 10 mL sample in the 50 mL glass beaker and mixed thoroughly. This was followed by the addition of 3 drops of 1% phenolphthalein as an indicator using a Pasteur pipette and mixed well. A 0.1 M sodium hydroxide (NaOH, Sigma-Aldrich) contained in the burette was carefully titrated into the mageu supernatant mixture until the mixture turned pink (Garner *et al.*, 2014; Rajkovic *et al*., 2007). The amount of NaOH used was recorded and the following formula was used to calculate the amount of titratable acidity:

$$Total\ titratible\ acidity = \frac{mL\ of\ solution \times 0.1\ M\ NaOH \times 7.5\ milliequivalent}{grams\ of\ sample}$$

3.3.5.3. Determination of sugar content in Degrees Brix

The total sugar of the mageu sample was measured using a refractometer (Magwaza and Opara, 2015). Two millilitres of the mageu were transferred into a 2 mL microcentrifuge tube and centrifuged at 10000 rpm for 5 minutes. Three drops of the supernatant were transferred to the refractometer while pointing directly to light and specific gravity (SG) was determined. The sugar content which is specified by the total soluble solids represented as degree brix (°Bx) was calculated from the specific gravity according to the formula below.

$$Degrees\ Brix\ (°Bx) = \frac{(SG - 1)}{0.004}$$

3.3.6. Sensory analysis of mageu

Sensory evaluation for all the mageu samples collected from day 0 of fermentation to day 4 was evaluated for the following characteristics: colour, smell, taste, texture, and consistency. The tasting panel was constituted by participants of different gender and age and were between 20 and 60 years of age. Participation was voluntary and all participants signed a consent form. The protocol received ethical clearance (Appendix A) from the Turfloop Research Ethics Committee (TREC) of the University of Limpopo. Papers and pens were used by the tasting participants to record their impressions of all samples of the mageu. Participants were given 10 mL of the different stages of fermentation contained in 20 mL clean clear cups closed tightly with the lid. The participants were given water, carrots, and cucumbers between tasting to avoid misinterpretation of tastes. Blind tasting was followed. Participants were not given any information about the product.

3.3.7. Shelf life of mageu

The shelf life of mageu was evaluated based on pH, total titratable acidity (TTA), sensory attributes (smell, colour, and consistency) after every week for 5 weeks. The pH was measured according to the method described in section 3.3.5.1 and TTA based according to the method in section 3.3.5.2 in this chapter. Sensory attributes were evaluated as outlined in section 3.3.6 above.

3.3.8. Statistical analysis

The results were interpreted using one-way ANOVA followed by Tukey's multiple comparisons of the GraphPad Prisms 8 Build-in analysis program. The significance difference was determined at $p \leq 0.05$. The parameters analysed were total microbial count (for LAB and yeast) and chemical parameters (°Bx, pH and TTA).

3.4. Results

Three recipes were chosen for evaluation of the microbiological, chemical properties, sensory profiling (colour, smell, taste, texture, and consistency), and shelf life of millet-based mageu based on the quantity of millet used for the preparation of mageu.

The results consist of microbial count for the LAB and yeasts for all the proportions as well as TTA, pH and °Bx. The microbial analysis included faecal and total coliform, colony morphology and cell morphology of LAB. Sensory profiling and shelf-life results are also presented.

3.4.1. Microbiological evaluation

Lactic acid bacterial count was increasing as fermentation progressed, while the yeasts were decreasing for all three proportions used for making mageu. Total and faecal coliforms were not detected.

In all the proportions of millet-based mageu, LAB were not detected on day 0 of fermentation and started to appear as fermentation started on day 1. However, a significant ($p < 0.05$) increase of LAB was observed from day 1 up to day 3 for mageu (9:1) (Figure 3.1 A) and the increase was significant until day 4 for mageu 8:2 and 5:5 (Figures 3.1 B and C).

The LAB count in millet-based mageu 9:1 ranged from $4.3 \times 10^5 – 10.2 \times 10^5$ CFU/mL, millet-based mageu 8:2 ranged from $7.2 \times 10^5 – 12.2 \times 10^5$ CFU/mL with millet-based mageu 5:5 ranging from $8.6 \times 10^5 – 20.0 \times 10^5$ CFU/mL. The mageu 5:5 had the highest LAB count compared to the two proportions which is followed by the mageu 8:2. The mageu with the highest amount of millet as inoculum had a higher count for LAB.

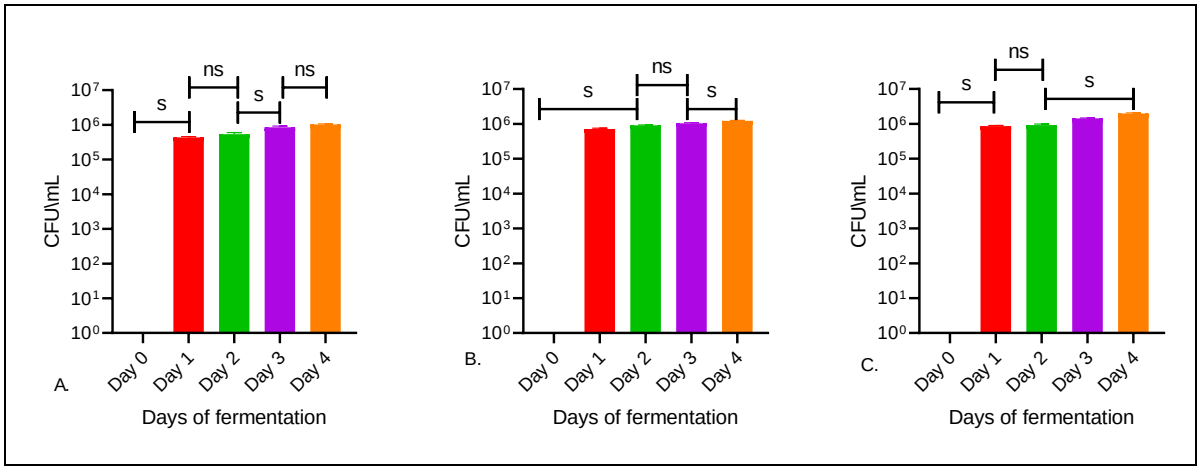

Figure 3.1: Total lactic acid bacteria count for all the millet-based mageu proportions, mageu 9:1 (A), mageu 8:2 (B) and mageu 5:5 (C). S implies statistical significance and ns is statistical non-significance.

The total yeast count in all the proportion of mageu was higher at day 0 and started to decrease from day 1 until last day of fermentation. A significant ($p < 0.05$) decrease was observed throughout fermentation period in mageu 9:1, mageu 8:2 and mageu 5:5 (Fig. 3.2).

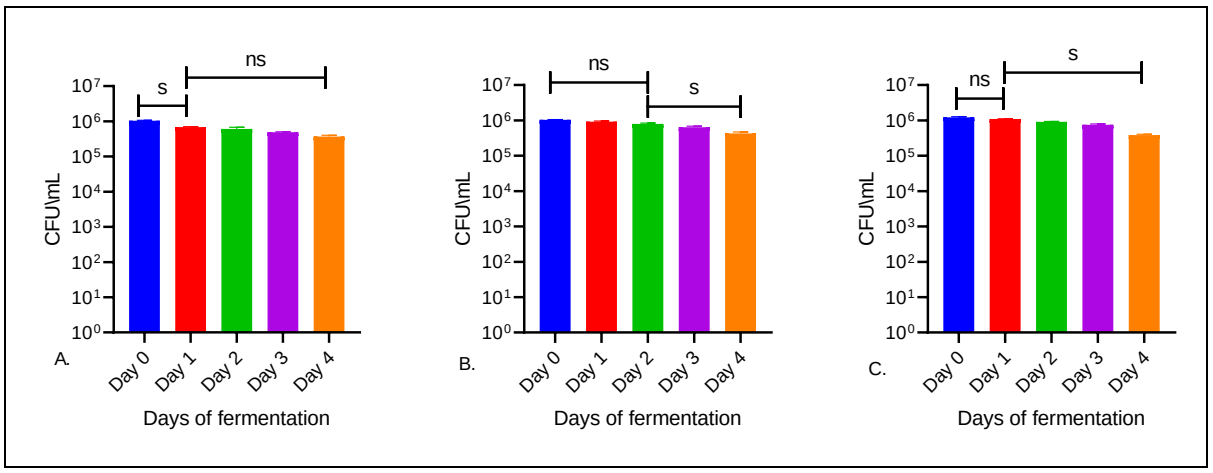

Figure 3.2: Total yeasts count in all the proportions of millet-based mageu, mageu 9:1 (A), mageu 8:2 (B) and mageu 5:5 (C). S implies statistical significance and ns is statistical non-significance.

The yeast count for millet-based mageu 9:1 was between $10.3 \times 10^5 – 3.7 \times 10^5$ CFU/mL, millet-based mageu 8:2 from $10.4 \times 10^5 – 4.3 \times 10^5$ CFU/mL and $12.1 \times 10^5 – 3.8 \times 10^5$ CFU/mL for millet-based mageu 5:5. Interestingly, the highest yeast count was noted on day 0 for mageu 5:5. Nonetheless, the count of yeast population are

higher than permissible limits set by the FDA.

3.4.2. Morphology of lactic acid bacteria isolates

Two different types of colonial morphology were observed from the fermenting mageu samples (Table 3.1).

Table 3.1: Cell and colony morphology of the isolates from the different mageu products.

Days of fermentation	**Isolates**	**Colony morphology**					**Cell morphology**	
		Shape	Size	Elevation	Margin	Colour	Gram staining	Cell shape
Day 1	**MD101**	Circular	Small	Raised	Filamentous	White	Positive	Bacillus
	MD102	Filamentous	Big	Umbonate	Entire	Cream white	Positive	Coccobacillus
Day 2	**MD101**	Circular	Small	Raised	Filamentous	White	Positive	Bacillus
	MD102	Filamentous	Big	Umbonate	Entire	Cream white	Positive	Coccobacillus
Day 3	**MD101**	Circular	Small	Raised	Filamentous	White	Positive	Bacillus
	MD102	Filamentous	Big	Umbonate	Entire	Cream white	Positive	Coccobacillus
Day 4	**MD101**	Circular	Small	Raised	Filamentous	White	Positive	Bacillus
	MD102	Filamentous	Big	Umbonate	Entire	Cream white	Positive	Coccobacillus

Two different isolates with similar Gram staining were observed for all the ratios of millet-based mageu from day 1 – 4. All the isolates were Gram-positive with the bacillus and coccobacillus cell shaped cells observed. The isolates differed in colour, margin, elevation, size and shape.

3.4.3. Biochemical characterisation of lactic acid bacteria

The biochemical properties of the two lactic acid bacterial isolates were similar except for the difference in sugar fermentation or glucose (Table 3.2).

Table 3.2: Growth characteristics of the lactic acid bacteria isolates.

Tests		**Isolate MD101**	**Isolate MD102**
Catalase		**-**	**-**
Salt tolerance	5%	**+**	**+**
	6.5%	**+**	**+**
	10%	**-**	**-**
Growth at different temperatures	25 °C	**+**	**+**
	37 °C	**+**	**+**
Motility test		**-**	**-**
Citrate utilisation		**-**	**-**
Sugar fermentation	Glucose	**+**	**-**
	Lactose	**+**	**+**
	Sucrose	**-**	**-**

The + sign denotes positive test and the - sign denotes the negative test.

The two LAB isolates demonstrated similar growth properties between them and in congruence to common lactic acid bacteria for characteristics of a such as ability to grow at low salt concentration, fermentation of sugars, catalase negative for anaerobic bacteria, citrate utilisation and motility.

3.4.4. Chemical profiling

3.4.4.1. pH

A significant ($p < 0.05$) decrease in pH was observed in all the millet-based mageu ratios (Fig. 3.3) throughout fermentation.

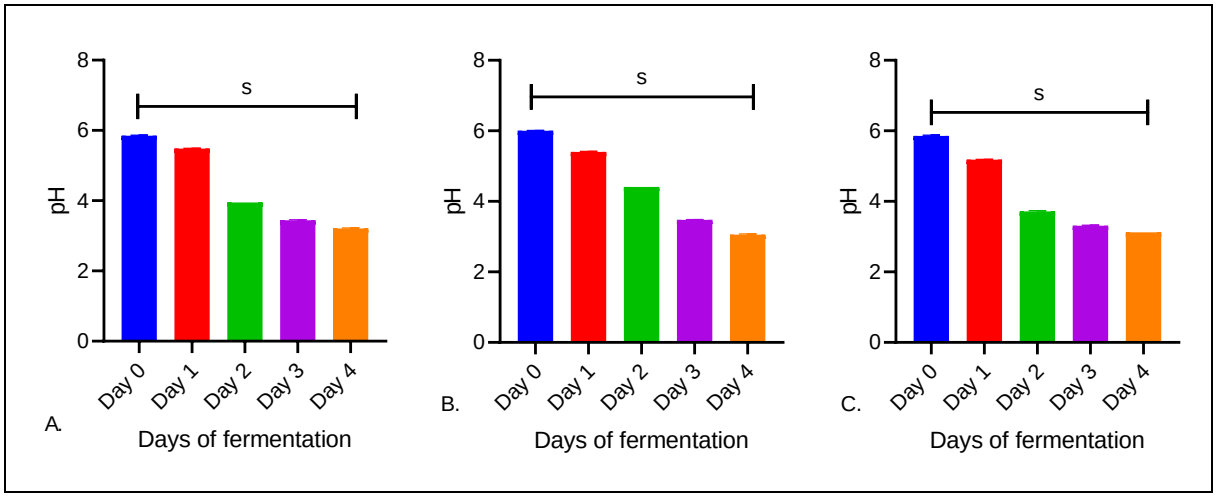

Figure 3.3: Profile of pH for all the millet-based mageu proportions,mageu 9:1 (A), mageu 8:2 (B) and mageu 5:5 (C). S implies statistical significance.

The pH of millet-based mageu 9:1 dropped from pH 5.9 to pH 3.2, millet-based mageu 8:2 from pH 6 to pH 3 while for millet-based mageu 5:5 the pH reduced from pH 5.9 to pH 3. Millet-based mageu 9:1 had a slightly higher pH on day 4.

3.4.4.2. Total titratable acidity

An increase in total titratable acidity was observed in all the millet-based mageu proportions, for millet-based mageu 9:1, a non-significant ($p > 0.05$) increase was observed within a range of 0.06 – 0.3% as indicated in figure 3.4. However, the significant ($p < 0.05$) increase and non-significant ($p > 0.05$) increase was observed in mageu 8:2 and mageu 5:5.

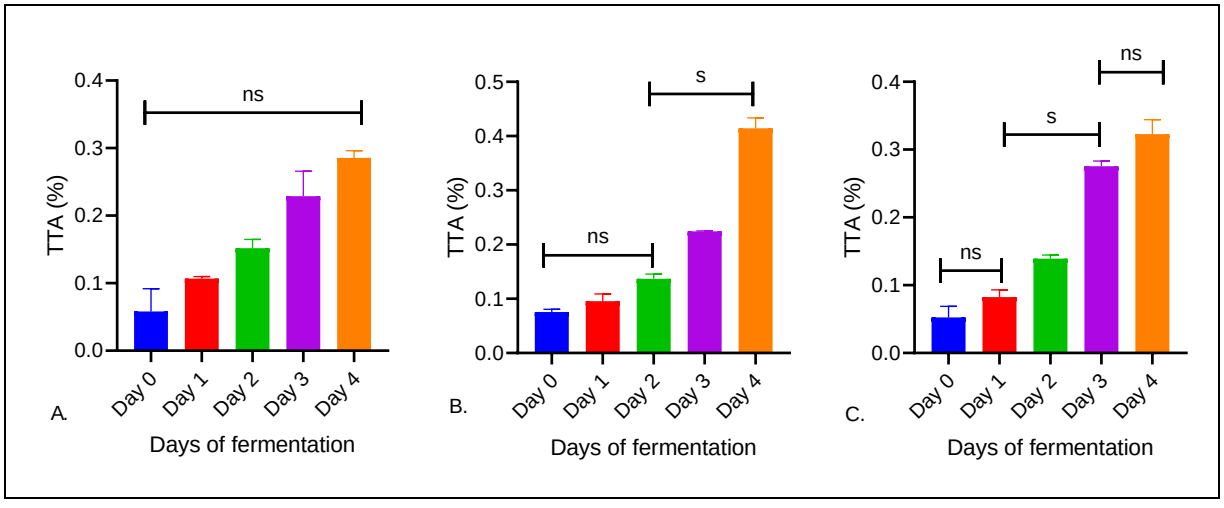

Figure 3.4: Total titratible acidity of millet-based mageu 9:1 (A), millet-based mageu

8:2 (B) and millet-based mageu 5:5 (C). S implies statistical significance and ns is statistical non-significance.

Millet-based mageu 8:2 ranged from 0.08 – 0.4% and millet-based mageu 5:5 ranged from 0.05 – 0.3%. The gradual increase in TTA (Fig 3.4) is inversely proportional to the steady drop in pH (Fig. 3.3).

3.4.4.3. Sugar content in Degrees Brix (°Bx)

All the unfermented gruels of millet-based mageu (Day 0) had low sugar content, however, a sharp increase was observed upon the start of fermentation on day 1 (Fig.3.5). A non-significant decrease ($p > 0.05$) in sugar content in all the fermenting mageu was observed on days 2 – 4.

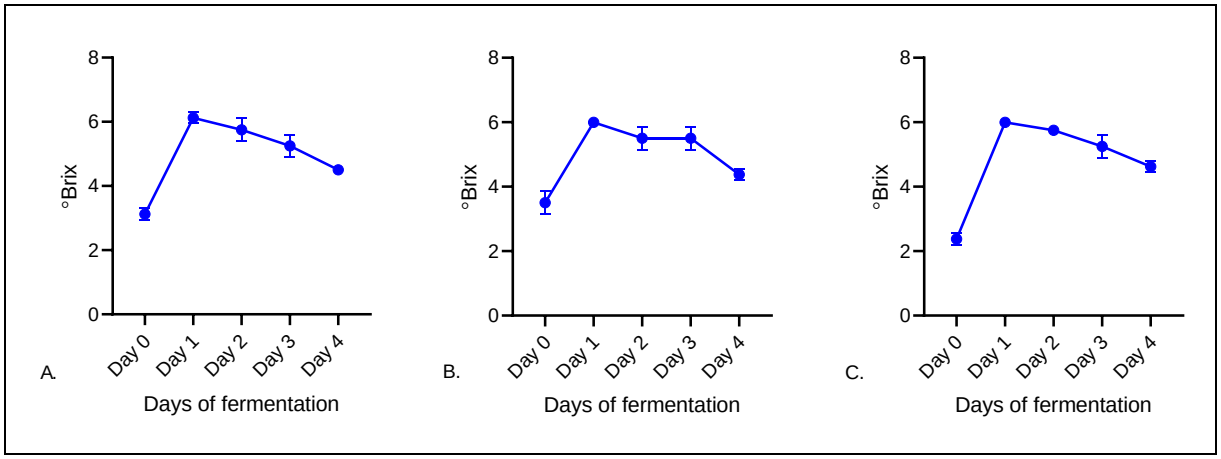

Figure 3.5: Sugar content in Degrees Brix (°Bx) in the different proportions of millet-based mageu, mageu 9:1 (A), mageu 8:2 (B) and mageu 5:5 (C).

Sugar content in millet-based mageu 9:1 ranged from 3.1 – 4.5 °Bx, 3.5 – 4.4 °Bx for millet-based mageu 8:2 and 2.4 – 4.6 °Bx millet-based mageu 5:5. Millet-based mageu 5:5 had a higher sugar content at the end of fermentation.

3.4.5. Sensory evaluation of the fermented brew

Tasting was conducted from the first day to the last day of fermentation by the panel. Colour, smell, taste, texture, and consistency were used to evaluate the three

proportions of mageu and descriptive comments and appreciation by the panellists were recorded.

Table 3.3: Sensory profiling of millet-based mageu 9:1.

	Fermentation period				
	Day 0	**Day 1**	**Day 2**	**Day 3**	**Day 4**
Colour	Brown	Brown	Light brown	Light brown	Creamy brown
Smell	Cooked maize meal	Cooked maize meal mixed with vinegar	• Cooked meal with vinegar • Cooked meal with achar	• Cooked meal with vinegar • Cooked meal with achar • Acrid	• Acrid • Cooked meal with achar and vinegar
Taste	• Cooked maize with raw millet • Cooked maize	• Raw-cooked millet with • Slightly sour taste	Slightly sour	Slightly sour	Slightly sour Sour
Texture	Rough	Rough	Slightly smooth	Slightly smooth	Smooth
Consistency	Thick	Thick	Moderately thick	Moderately thick	Moderately thick

Mageu 9:1 generally tasted sour, with a vinegary smell during fermentation. This mageu preparation became slightly more liquid and lighter in colour with progression of fermentation period (Table 3.3). The creamy brown colour and the smooth texture were appreciated.

Table 3.4: Sensory profiling of millet-based mageu 8:2.

	Fermentation period				
	Day 0	**Day 1**	**Day 2**	**Day 3**	**Day 4**
Colour	Brown Dark brown	Brown	Light brown	Light brown	Light brown
Smell	• Raw maize meal • Wet grains	Acidic smell	Acidic smell	Highly acidic smell	Highly acidic smell
Taste	Raw watery millet	• Slightly sour • Cooked maize	Slightly sour	Sour	Very sour
Texture	Rough	Rough	Slightly smooth	Slightly smooth	Smooth
Consistency	Thick	Thick	Moderately thick	Moderately thick	Moderately thick

The organoleptic properties of the fermenting mageu were the same at days 3 and 4 except for the texture that changed from slightly smooth on day 3 to smooth on day 4, taste from sour on day 3 to very sour on day 4. The smooth texture was preferred, it was recorded as palatable on days 3 and 4 of fermentation, however, the participants did not like the taste (Table 3.4). The sour taste was matched with an acidic smell.

The sensory attributes for mageu 5:5 were varied throughout fermentation, especially for taste and texture characteristics (Table 3.5).

Table 3.5: Sensory profiling of millet-based mageu 5:5.

	Fermentation period				
	Day 0	**Day 1**	**Day 2**	**Day 3**	**Day 4**
Colour	• Brown • Light brown	Light brown	Light brown	Creamy brown	Creamy
Smell	Raw maize	Raw maize with less acidic smell	Less acidic smell	Acidic smell	Acidic smell
Taste	Soaked maize	• Slightly sour • Soaked maize	Slightly sour-sweet	• Slightly sour • Sweet-sour	• Sweet sour • Sweet
Texture	Rough	Slightly rough	Slightly rough	Slightly smooth	Smooth
Consistency	Thick	Thick	Slightly thick	Slightly thick	Slightly thick

Mageu 5:5 was found to be palatable on day 4 of fermentation, the taste was deemed sweet-sour and sweet, and the smell was generally described as acidic. The tasting panel preferred millet-based mageu 5:5 in all the attributes compared to millet-based mageu 9:1 and millet-based mageu 8:2.

3.4.6. Shelf-life of the millet-based mageu

The shelf life of mageu inferred stability in the sensory parameters and selected chemical properties over a set period of storage, hence its storage potential. The stability of mageu preparations was evaluated for pH, TTA, smell, colour, and consistency on weekly intervals during a 5-week storage period and the changes were noted.

3.4.6.1. pH of millet-based mageu during storage

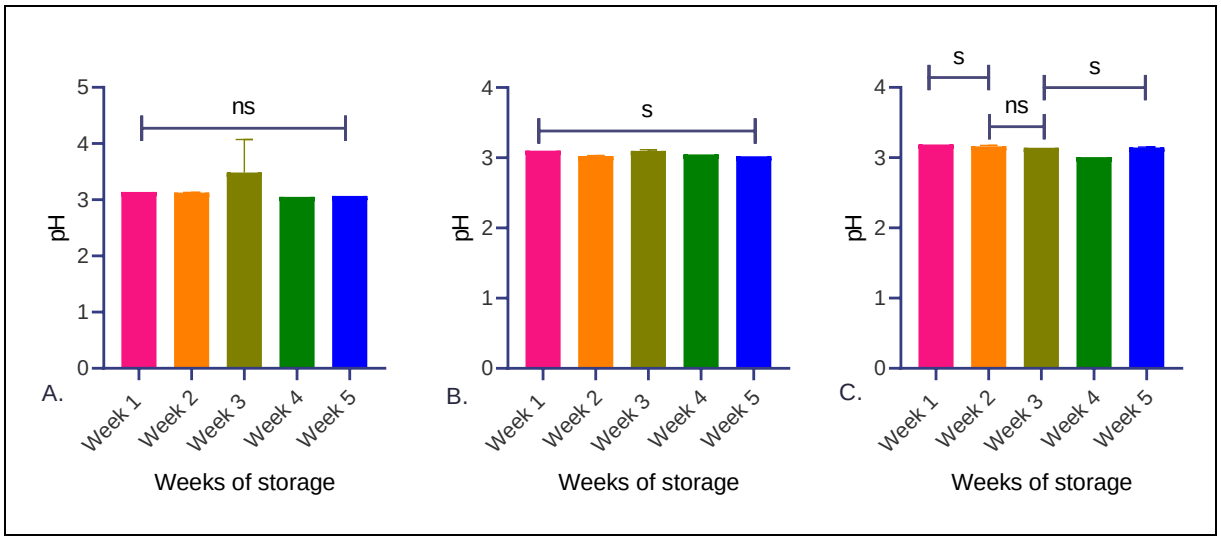

Figure 3.6: Changes in pH of all the proportions during period of storage, millet-based mageu 9:1 (A), millet-based mageu 8:2 (B) and millet-based mageu 5:5 (C). S implies statistical significance and ns is statistical non-significance.

The pH of mageu proportions during storage dropped as compared to the pH during the fermentation period on figure 3.6. Mageu 9:1 dropped from 3.14 – 3.02, while mageu 8:1 dropped from 3.10 down to 3.02 and mageu 5:5 from 3.19 – 3.15.

3.4.6.2. Total titratable acidity of millet-based mageu during storage.

The total titratable acidity of all the proportions of mageu during fermentation (Fig 3.4) was lower as compared to the TTA during the storage period (Fig 3.7).

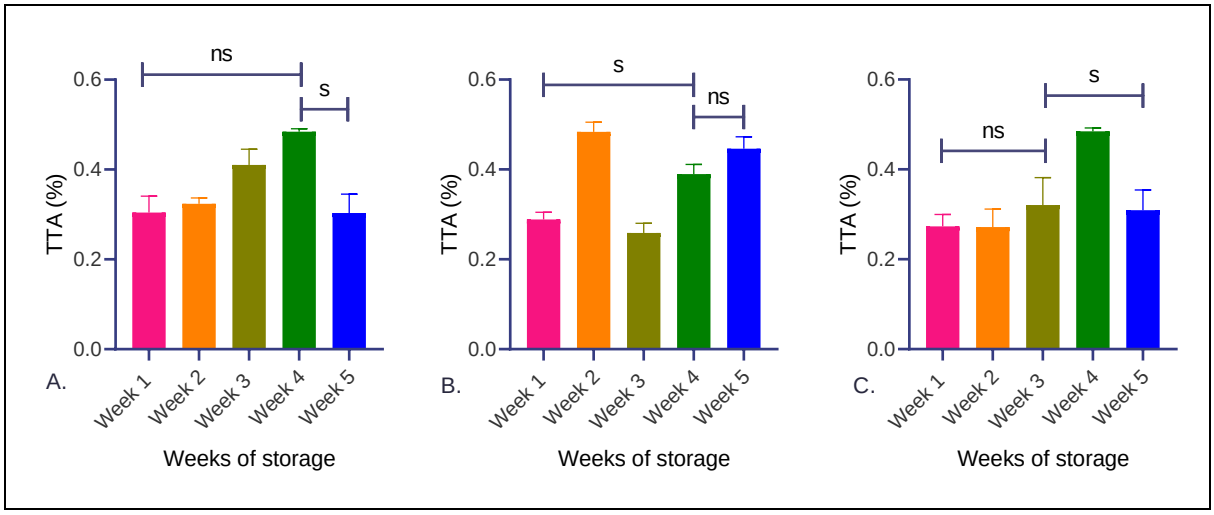

Figure 3.7: Total titratable acidity of millet-based mageu during weeks of storage, mageu 9:1 (A), mageu 8:2 (B) and mageu 5:5 (C). S implies statistical significance and ns is statistical non-significance.

A higher increase in the TTA of the mageu proportions was observed (Fig 3.7). The millet-based mageu 9:1 raised from 0.60 – 0.61%, millet-based mageu 8:2 increased from 0.29 – 0.4%, and millet-based mageu 5:5 raised from 0.27% up to 0.62%.

3.4.6.3. Sensory analysis of millet-based mageu proportions during storage

The organoleptic attributes of the fermented mageu proportions changed slightly during storage period (Table 3.6) when compared to the last day of fermentation period (day 4) (Table 3.3 – 3.5) just before the storage period could commence.

Table 3.6: Changes in organoleptic properties of all the millet-based mageu proportions (mageu 9:1, mageu 8:2 and mageu 5:5) after 5 weeks of storage.

Weeks of storage	Millet-based mageu 9:1			Millet-based mageu 8:2			Millet-based mageu 5:5		
	Colour	**Smell**	**Consistency**	**Colour**	**Smell**	**Consistency**	**Colour**	**Smell**	**Consistency**
Week 1	Creamy brown	Acrid	Moderately thick	Light brown	Highly acidic smell	Moderately thick	Creamy brownish milk	Acidic smell	Slightly thick
Week 2	Creamy brown	Acrid	Moderately thick	Light brown	Highly acidic smell	Moderately thick	Creamy brownish milk	Acidic smell	Slightly thick
Week 3	Creamy brown	Acrid	Moderately thick	Light brown	Highly acidic smell	Moderately thick	Creamy brownish milk	Acidic smell	Slightly thick
Week 4	Creamy brown	Acrid	Moderately thick	Light brown	Highly acidic smell	Moderately thick	Creamy brownish milk	Acidic smell	Slightly thick
Week 5	Creamy brown	Acrid	Moderately thick	Light brown	Highly acidic smell	Moderately thick	Creamy brownish milk	Acidic smell	Slightly thick

The smell and consistency of mageu 8:2 (highly acidic smell and moderately thick) and mageu 5:5 (acidic smell and slightly thick) during storage were the same when compared to the last day of fermentation (Tables 3.4 and 3.5) except for the colour of mageu 5 that changed to creamy brownish milk from creamy (Table 3.5). The panellist showed similar likings to the acidic smell of mageu 9:1 throughout the storage days.

3.5. Discussion

Millet is available in abundance, has nutritious health benefits as well as the ability to improve flavour during fermentation (Sarita and Singh, 2016). Mageu is a non-alcoholic drink, which often serves as a meal on its own. Three different mageu products were produced in this study, each made from a combination of millet flour and maize meal at different proportions. The sensory properties of the millet are contributed by the raw materials (Maize and millet in this study), the activity of fermenting microorganisms and their metabolites (Mashau *et al*., 2020).

The presence and population sizes of LAB, yeasts, faecal coliform as well as total coliform were investigated. LAB and yeasts are important for fermentation and thus product development, while the coliforms are analysed for the safety of the product. According to the food and drug administration (FDA), the presence of indicator microorganisms and food pathogens such as *Escherichia coli*, *Salmonella* sp. faecal and total coliform are prohibited in different food products (Leone, 2014). The absence of coliforms in mageu products prepared in this study was indicative of the microbiological safety of the products for human consumption. It could also imply that all the hygienic protocols were followed to avoid the presence of contaminants (Mashau *et al*., 2020).

Microorganisms such as yeasts are known to initiate fermentation thereby providing all the necessary nutrients to other microorganisms present in the fermenting product, as well as lowering the pH (Laranjo *et al*., 2019). This is supported by the observed rising yeasts count in the early days of mageu fermentation (days 0 – 1). In contrast, LAB were not detected in the unfermented gruel (Day 0) of millet-based mageu. LAB are non-motile and do not require oxygen for growth (Gebreselassie *et al*., 2016). LAB can tolerate acid and their major fermentation product is lactic acid (Maqsood *et al*.,

2013). It could be that LAB start to grow once fermentation has started and the conditions (such as decreasing pH) are suitable for their growth (Simatende *et al*., 2019). The decrease in the yeasts count and the increase in the LAB count could be due to the changes in the environment that favour the LAB thereby highlighting the LAB as the main drivers of mageu fermentation. Moreover, LAB may be producing organic acids, bacteriocins and hydrogen peroxide that harm the yeast population (Laranjo *et al*., 2019) and other undesirable bacteria, and these organisms may be competing for the nutrients (Ponomarova *et al*., 2017). Noting that the proportions of millet-based mageu differed significantly ($p < 0.05$) with the LAB and yeast population, it could be that the amount of millet flour used as inoculum to initiate fermentation played a crucial role because millet-based mageu 5:5 had the highest count of LAB bacteria.

Several studies on mageu production have reported similar observations as the current study whereby the decrease in yeasts population was concurrent with the increase in the LAB population, and pathogens were not detected (Fadahunsi and Soremekun, 2017; Idowu *et al*., 2016; Salvado *et al*., 2016). The lactic acid bacteria commonly identified in maize based mageu are *Lactococcus lactis*, *L. bulgaricus* and *L. brevis* (Fadahunsi and Soremekun, 2017; Idowu *et al*., 2016; Salvado *et al*., 2016) and they all belong to the *Lactobacillus* genus.

LAB isolates coded as MD101 and MD102 were bacillus and coccobacillus shaped and Gram-positive, indicative of LAB (Ismail *et al*., 2018). The biochemical results confirmed that the two isolates, MD101 and MD102 obtained belonged to the LAB group. LAB cannot use citrate as the energy source due to their inability to ferment it although they can ferment different sugars and utilise them as the energy source (Maakelo *et al*., 2021). These isolates are osmotolerant since they can tolerate different NaCl concentration (Maakelo *et al*., 2021). This allows LAB to produce lactic acid in the gut in the presence of various salt concentrations (Maakelo *et al*., 2021). On the other hand, the ability of LAB to grow at 37 °C may promote a positive impact towards the health of individuals. Moreover, LAB could assist to introduce good bacteria (probiotics) in the human gut thus reducing the effect of harmful substances and infection (Maakelo *et al*., 2021).

Factors such as nutrients, acidity, pH, sulfite, and ethanol play an important role towards the metabolic activities and growth of LAB (Wang *et al*., 2021). According to Maakelo *et al*. (2021) and Mashau *et al*. (2020), the pH of mageu ranges between 3.0 and 4.5, thus it corroborates with the findings of this study**.** The decrease in pH is the result of the presence of LAB producing lactic acid, hence the inverse relationship between the TTA and pH. The inverse proportionality between TTA and pH was reported by Idowu *et al*. (2016) on the production and nutritional evaluation of mageu. The TTA, which refers to the organic acids in a product, plays a significant role towards the flavour of the fermented products while the pH outlines the level of acidity of the product (Harris, 2016). It can be deduced that the production of organic acids in the fermenting mageu leads to a lower pH, which inhibits other microorganisms and allows LAB to thrive due to their tolerance to acidic conditions and a growth range at lower pH (Abbasiliasi *et al*., 2017). Most food pathogens cannot survive at low pH conditions (Maakelo *et al*., 2021). This is beneficial towards the shelf life of mageu.

The changes in sugar content (°Bx) may be due to the action of the amylolytic enzyme activities which breaks down starch into glucose monomers (Idowu *et al*., 2016) or such observation could be resulting from incomplete starch degradation during fermentation (Maakelo *et al*., 2021). Microorganisms often use sugar (°Bx) as their substrate during food fermentation (Nkhata *et al*., 2018). The balance between the total titratable acidity and the °Bx content bring about the unique flavour of fermented products (Abbasiliasi *et al*., 2017), hence the sour, sweet, and sweet-sour tastes observed by the tasting panel members.

Sensory profiling entails the evaluation of taste, smell, texture, consistency, and colour of mageu (Boyiako *et al.*, 2020). Temperature used during fermentation and the fermentation period all contribute to the sensory characteristics of a product. The changes in temperature and period of fermentation, influences the brown pigment of millets and the tannin content of millets (Dayakar *et al*., 2017) resulting in changes in the colour of millet-based mageu. It may also indicate that the fermentation period and high fat content in millets improved the texture of mageu as well as influenced the colour (Kumar *et al*., 2021; Sarita and Singh, 2016). Although the sour-bitter taste in millet-based mageu could be attributed to the organic acids produced during lactic

acid fermentation (Mashao *et al*., 2020), the saponin coating of millet which gives it its natural bitter taste (Kumar *et al*., 2021; Sarita and Singh, 2016) should also be considered.

Lactic acid bacteria have bio preservative properties and with their ability to produce antimicrobial compounds, they could increase the stability of fermented products and reduce the occurrence of food borne diseases (Abbasiliasi *et al*., 2017). During the storage of mageu at 4 °C the pH and TTA of the product was not constant but remained within the common pH range of mageu which is 3.0 to 4.5 and TTA that ranged from 0.05% to 0.6% (Maakelo *et al*., 2021; Mashau *et al*., 2020). The organoleptic properties such as consistency, smell and colour were not affected by the storage period.

At the end of sensory evaluation, millet-based mageu 8:2 became less favourable to the tasting panel due to the highly acidic smell and strong sour taste.

Fermentation of millet flour for production of mageu improved the availability of LAB, reduced the natural bitter taste of millet and overall produced a mageu product with unique sensory attributes to common mageu products produced from either maize or sorghum. Two proportions of millet-based mageu, mageu 9:1 and mageu 5:5 were considered for further analysis. The proportions were chosen based on the sensory acceptability of the fermented mageu as they were perceived as palatable.

CHAPTER 4. EVALUATION OF THE MICROBIOLOGICAL PROPERTIES, PROBIOTIC AND SAFETY POTENTIAL OF LACTIC ACID BACTERIA

4.1. Introduction

Mageu is a naturally fermented non-alcoholic drink commonly made from one or a combination of different cereals and it is consumed by all age groups for anytime meal. It has elevated levels of lactate producing bacteria which are provided by the type of cereal used as an inoculum (Boyiako *et al*., 2020) and it is an important food product in the weaning of infants. Mageu is fermented spontaneously at an ambient temperature for about four days (Eswatini, 2019). Maize meal, leftover maize porridge and sorghum are commonly used in Botswana, Zimbabwe and South Africa as starter material for production of mageu (Idowu *et al*., 2016).

Conditions such as period and temperature of fermentation, storage and raw materials used (maize, wheat or sorghum) are monitored during production of mageu since they all affect the amount of different microorganisms produced, especially lactic acid bacteria (LAB) which are considered as probiotics. Oh and Jung (2015) suggested that for LAB bacteria to be termed probiotics, which are live microorganisms that plays a significant role towards the health of humans, they must survive the acidic conditions and express a high tolerance of bile conditions of the stomach. Interestingly, mageu has probiotic properties just like other non-alcoholic beverages such as yoghurt, ting and ragi due to the presence of LAB (Salvado *et al*., 2016). Anitha *et al*. (2018) documented the safety and probiotic characteristics of LAB from fermented ragi.

Importantly, rich sources of LAB include plant materials and carbohydrate substrates (Hassanzadazar *et al*., 2012). LAB were previously isolated from maize-based mageu, while moulds and yeasts were sporadically observed (Salvado *et al*., 2016). LAB are the predominant microflora in mageu fermentation, and the observed species include *Lactococcus lactis*, *L. bulgaricus* and *L. brevis* (Salvado *et al*., 2016). The LAB play a role in the digestion of starch to lactic acid and other organic acids that lead to

the reduction in pH (Salvado *et al*., 2016). The low pH is beneficial for the safety of mageu by inhibiting the growth of harmful and undesirable microorganisms (Maakelo *et al*., 2021).

Mageu is dominantly produced from maize meal (Eswatini, 2019) while cereals, such as millet, are not considered due to its bitter taste and this has limited exploration of microbial population which could be present in the millet-based mageu. This study explored the use of pearl millet flour in the production of mageu and the characterisation of the isolated LAB strains.

4.2. Materials and methods

4.2.1. Preparations of millet-based mageu and sample collection

Two proportions of millet-based mageu, described below, were prepared in the total volume of 7 L following a modified method of Fadahunsi and Soremekun (2017).

i. Mageu 9:1 (80 millet flour + 10 maize meal:10 millet flour)
ii. Mageu 5:5 (25 millet flour + 50 maize meal: 25 millet flour)

For 9:1 proportion, 800 g of pearl millet flour and 100 g of maize meal were mixed in a bowl followed by addition of 1 L of distilled water and mixed thoroughly to make a paste. Thereafter, 4 L of tap water was bo iled in a pot and the paste was added slowly with stirring and cooked to bubbling point for 25 minutes, then heat was reduced to moderate temperature (~50 °C) for 5 minutes to obtain the soft porridge. The mixture was stirred carefully after every 5 minutes to achieve a uniform mixture of soft porridge during the process of cooking.

Following cooking, the soft porridge was aseptically transferred into a clean 9 L plastic bucket and allowed to cool at ambient temperature. The millet soft porridge was mixed with 100 g millet flour and 2 L distilled water to produce a gruel for mageu production. The ingredients were mixed thoroughly until a uniform mixture was obtained. A sample was taken aseptically using sterile 50 mL centrifuge tubes and labelled as day 0 (before the fermentation process started). The bucket was then closed tightly and

allowed to ferment naturally at 25 °C for four days. On day 4 of fermentation, a 50 mL sample was also taken after mixing thoroughly and labelled as such. The bucket was then stored at 4 °C and samples were taken aseptically in 7 days interval for a period of 2 months (8 weeks) using the sterile 50 mL centrifuge tubes. The samples were stored at -20 °C after preparation of microbial stock cultures.

The same procedure was followed for the 5:5 proportion using the amount of millet and maize as indicated in the ratio and the amount of water used was the same throughout. For this proportion, 250 g of millet flour was mixed with 500 g of maize meal along with 250 g of millet flour as an inoculum.

4.2.2. Preparation of microbial stock cultures

Thirty millilitres (30 mL) of the collected samples were aseptically transferred into sterile 50 mL centrifuge tubes. The samples were then centrifuged at 10000 rpm for 5 minutes.

To prepare the stock cultures, 2 mL of the supernatant of mageu sample were transferred into sterile 2 mL microcentrifuge tubes and centrifuged for 10 minutes at 5000 rpm. The supernatant was discarded, and 2 mL of a 50% glycerol solution (prepared in 0.85% saline) was added to the pellet, mixed thoroughly, and stored at minus 20 °C. All preparations were done in triplicates.

4.2.3. Microbiological analysis

4.2.3.1. Enumeration of yeasts/ moulds, total bacteria and *Escherichia coli*

Enumeration of yeasts and moulds, total bacteria (Aerobic count), and *E. coli* was performed in duplicates using the TEMPO system procedure (Cochran, 1950) at Limpopo Agro-Food Technology station (LATS, University of Limpopo). Hundred microlitres (100 µL) of the stock culture sample was aseptically mixed with 900 µL of peptone water in a 2 mL microcentrifuge tube. The contents were mixed thoroughly and transferred into a sterile glass vial. Thereafter, 3 mL of sterile distilled water was added to the mixture in the glass vial and mixed thoroughly. The manufacturer's instructions were followed for loading, incubation, and analysis of the samples. For the

yeasts and moulds, the cards were incubated for 72 hours at 25 °C whereas the cards for total bacteria and *E. coli* were grouped together and incubated at 30 °C for 24 hours. The TEMPO reader software calculates the results based on Most Probable Number (MPN) and the results were presented in colony forming unit per millilitres (CFU/mL).

4.2.3.2. Isolation and enumeration of lactic acid bacteria

Isolation and enumeration of LAB from the mageu samples was performed following the culture dependent method. The 100 µL of the stock culture was mixed thoroughly with 900 µL of 0.85% saline solution. Serial dilutions from 10^{-1} to 10^{-6} were prepared. De Man Ragosa and Sharpe agar medium (MRS; Sigma-Aldrich) was used for isolating LAB. The 100 µL of the dilutions was spread plated on MRS agar using sterile 5 mm sterile beads. The procedure was performed aseptically in triplicates. The plates were incubated anaerobically for 48 hours at 37 °C.

Following incubation, the colonies on the MRS plates were counted manually. Only plates with colony counts in the range of 30 – 300 were considered. The results were noted as colony-forming units per millilitre (CFU/mL) as shown in the equation below.

$$CFU/mL = \frac{number\ of\ colonies \times dilution\ factor}{volume\ plated}$$

4.2.4. Purification of lactic acid bacteria isolates

Different colonies on the MRS agar were randomly chosen based on colour, shape, margin, elevation, and size. Gram staining was performed, and cell morphology and staining reaction were recorded.

Purification of LAB was performed by sub-culturing on the same culture medium following the Gram staining procedure. The selected LAB isolates were streaked repeatedly on MRS agar and incubated anaerobically at 37 °C for 48 hours until pure cultures were obtained. The pure cultures were stored at 4 °C.

4.2.5. Biochemical characterisation of lactic acid bacteria

Pure cultures of LAB isolates were characterised using various biochemical tests.

4.2.5.1. Catalase test

The catalase test for the isolated LAB was determined according to Chapter 3, section 3.3.4.1.

4.2.5.2. Salt tolerance

Salt tolerance of the isolates was determined as outlined in Chapter 3, section 3.3.4.2. with minor modifications such as incubating not only at 37 °C but also at 4 °C and 25 °C.

4.2.5.3. Growth test at different temperatures

Growth temperature assay of the isolated LAB was measured as described in Chapter 3, section 3.3.4.3. with modifications of adding another incubation temperature of 4 °C to the temperatures of 25 °C and 37 °C.

4.2.5.4. Motility test

Sulphur, indole and motility agar (SIM; Sigma-Aldrich) slants were prepared according to the manufacturer's instructions. A sterilised inoculating needle was used to inoculate the middle of the agar. The tubes were incubated for 7 days at 37 °C while observing the growth by checking the diffuse zones showing growth from the inoculation line (Ismail *et al*., 2018). *E. coli* (ATCC 25992) was used as the positive control.

4.2.5.5. Citrate utilisation

Simmons citrate (Sigma-Aldrich*)* agar slants were prepared in test tubes according to the instructions of the manufacturer. All the cultures were transferred into the tubes by streaking on the slants. The tubes were incubated aerobically for 7 days at 37 °C to observe the colour change from green to blue, indicative of the ability to use citrate as

an energy source (Ismail *et al*., 2018). *Klebsiella pneumoniae* (ATCC 10102) was used as the positive control.

4.2.5.6. Sugar fermentation

One gram (1 g) of lactose, sucrose and glucose were separately added to a solution containing 1,6 g nutrient broth, 1 g peptone, 0.3 g meat extract, 0.5 g NaCl, 100 mL distilled water and 0.008 g phenol red (pH indicator) in test tubes. Durham tubes were added to the test tubes for determining the fermentative ability of the cultures. Cultures were used to inoculate the different sugar media and were incubated at 37 °C for 24 hours, with *E. coli* (ATCC 25992) as a positive control. The presence of bubbles in the inverted Durham tubes was taken as a positive result for fermentation (Ismail *et al*., 2018).

4.2.5.7. Nitrate reduction test

Nitrate reduction for the chosen isolates was carried out according to Adeyemi and Beckley (1986) with *P. aeruginosa* (ATCC 9027) as a positive control. Cultures not older than 24 hours were inoculated into the nitrate broth (Sigma-Aldrich) and incubated at 37 °C for 24 hours. Following incubation, 8 drops of Sulfanillic acid were added slowly followed by 8 drops of α-naphthylamine. The reaction was observed for colour development. In cases where there was no colour development after addition of sulfanillic acid and α-naphthylamine, zinc dust was added, and observation of colour change was done after 3 minutes. A positive test was recorded for the appearance of cherry red colour while no development of cherry red after addition of zinc dust was noted as a negative result.

4.2.5.8. Oxidase test

The oxidase test for the selected isolates was done according to the method of Adeyemi and Beckley (1986) using the direct plate method. *Pseudomonas aeruginosa* (ATCC 9027) was used as the positive control. The isolates were streaked aseptically on the nutrient agar (Sigma-Aldrich) and incubated for 48 hours at 37 °C. Thereafter, 3 drops of Kovac's oxidase reagent (Sigma-Aldrich) were added slowly on top of the isolates while observing any colour change within 10 seconds. The appearance of

either a deep blue or purple colour was noted as a positive result while no colour change denoted a negative result.

4.2.5.9. Blood agar test

Blood agar test follows the Bergey's Manual of Systematic Bacteriology to detect haemolysis by cytolytic toxins secreted by any of the isolates (Adeyemi and Beckley, 1986). The method was followed with minor modifications. Blood agar medium was supplemented with 7% filtered chicken blood. Selected liquid cultures were prepared in MRS broth and incubated using a shaking incubator for 48 hours at 37 °C. One hundred microlitres (100 µL) of the liquid cultures was then transferred to the blood agar plates and spread thoroughly using sterile beads. The plates were incubated at 37 °C for 24 hours and afterwards notable zones around colonies were used to check haemolysis of the isolates. *Enterococcus faecalis* (ATCC 29212) was used as the positive control.

4.2.6. Identification of lactic acid bacteria

The identification of Lactic acid bacteria was outsourced to Inqaba Biotechnical Industries Pty (Ltd). LAB identification was done following sequencing of the 16S rRNA gene (Altschul *et al*., 1997). Genomic DNA of the selected isolates was extracted using Quick DNA kit (Bacterial Miniprep Kit) and the 16S rDNA target region was amplified using 16S-27F and 16-1492R primers (Turner *et al*., 1999; Weisburg *et al*., 1991). The PCR amplicons were visualised on agarose gel (1%) and further purified using the ExoSAP procedure. The amplicons were purified and sequenced in forward and reverse direction. Furthermore, the identity of organisms was determined through homology analysis of sequences obtained in the lab and those from the databases in National Centre for Biotechnology Information (NCBI). Matches above 99% threshold were considered for sequence identification.

4.2.7. Probiotic properties of lactic acid bacteria

4.2.7.1. Acid tolerance

The selected isolates were evaluated for acid tolerance following the method of Oh and Jung (2015) with minor modifications. The isolates were incubated for 48 hours in

MRS broth using a shaking incubator (200 rpm) set at 37 °C. MRS broth supplemented with 1% pepsin was prepared and transferred to different blue or Bjorn bottles whereby each bottle was adjusted to a different pH of either 1.5, 2, 2.5 or 3 using 10 M hydrochloric acid (HCl) and to pH 7 using 0.1 M NaOH (used as control). One millilitre of the liquid culture (of the isolates) was transferred into the pepsin supplemented broths of adjusted pH and the control. Reference samples for viable count were taken aseptically and plated on MRS agar medium prior to incubation. At 3, 24 and 36 hours of incubation, 100 µL of samples were collected and plated on MRS agar medium and incubated anaerobically at 37 °C for 48 hours. Following incubation, the colonies were enumerated and used to calculate the survival rate using the formula below.

$$Survival\ rate\ (\%) = \frac{cell\ number\ \left(\frac{logCFU}{mL}\right) survived\ in\ MRS\ containing\ HCl}{cell\ number\ \left(\frac{logCFU}{mL}\right) of\ initial\ inoculated\ cell} X\ 100$$

4.2.7.2. Bile salt tolerance

The bile tolerance of the LAB isolates was evaluated according to the method of Oh and Jung (2015) with minor modifications. The isolates that survived in different pH values were further tested for survival in bile conditions using bile salts. The MRS broth was prepared and supplemented with 0.3% and 0.9% of sodium deoxycholate (Sigma-Aldrich). After 3, 24 and 36 hours of incubation, the test organisms were further incubated in MRS supplemented with sodium deoxycholate for 24 – 48 hours in a shaking incubator (200 rpm) at 37 °C. Following incubation, the bile salt tolerances of the isolates was assessed by growing the incubated isolates in MRS agar plate medium anaerobically for 48 hours at 37 °C. The colonies were counted, and the survival rate was calculated using the formula below.

$$Survival\ rate\ (\%) = \frac{cell\ number\ \left(\frac{logCFU}{mL}\right) survived\ in\ MRS\ containing\ SD}{cell\ number\ \left(\frac{logCFU}{mL}\right) of\ initial\ inoculated\ cell} X\ 100$$

SD=Sodium deoxycholate

4.2.8. Safety properties of lactic acid bacteria

4.2.8.1. Antibiotic susceptibility test

The Kirby-Bauer Disk Diffusion Susceptibility method was used to test the antibiotic susceptibility of the selected isolates (Bauer *et al*., 1959). Antibiotic discs containing 30 µg chloramphenicol, 10 µg penicillin, 10 µg streptomycin, 30 µg tetracycline and 30 µg vancomycin were used. The isolates were inoculated in MRS broth and incubated in the shaking incubator at 37 °C for 48 hours. Thereafter, the liquid cultures were spread on Mueller-Hinton agar plates using sterile cotton balls and the antibiotic disks were placed on the plates using a sterilised forceps. The plates were incubated at 37 °C for 24 hours. Sizes of the clear zone around the discs on the plates were measured and recorded in millimetres (mm), and further used to determine whether the LAB isolates are sensitive, intermediate and resistance towards the selected antibiotics following the National Committee for Clinical Standards (NCCLS) (1990) in table 4.1.

Table 4.1: Zone sizes (mm) interpretation standards for lactic acid bacteria.

Antibiotics disk content in µg	Diameter of zones of inhibitions to the nearest mm		
	Resistance	Intermediate	Susceptible
Chloramphenicol (30 µg)	≤ 12	13 – 17	≥ 18
Penicillin (10 µg)	≤ (24 – 30)	---	≥ 33
Streptomycin (10 µg)	≤ 11	12 – 14	≥ 15
Vancomycin (30 µg)	≤ 9	10 – 11	≥ 12
Tetracycline (30 µg)	≤ 14	15 – 18	≥ 19

4.2.8.2. Antioxidant activity of the selected lactic acid bacteria

The antioxidant activity of the isolates was determined using the 2,2-Diphenyl-1-pycrilhydrazil (DPPH) assay following the modified method of Oh and Jung (2015). Liquid cultures of selected isolates were prepared in MRS broth and incubated for 48 hours using shaking incubator (200 rpm) at 37 °C. After incubation, 1 mL of the isolates was transferred into clean test tubes following the addition of 1 mL of a 0.4 mM DPPH solution. The L-ascorbic acid was prepared similarly and used as the standard whereas the control was prepared by adding 1 mL of MRS broth to 1 mL of DPPH solution in a test tube. The mixture was allowed to react by incubating it for 30 minutes

in the dark. The antioxidant activity was determined by measuring the absorbance at a wavelength of 517 nm. Preparations were performed in triplicates. The DPPH radical scavenging effect was determined according to the formula below.

$$Scavenging\ activity\ (\%) = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control}\ X\ 100$$

4.2.9. Statistical analysis

The results were interpreted using one-way ANOVA followed by Tukey's multiple comparisons of the graph pad prism 8 after testing for normality of the data. Significant difference was determined at $p \leq 0.05$. Microsoft excel was used to calculate the average and standard deviation of antibiotic susceptibility and antioxidants activity. Experiments were conducted in replicates.

4.3. Results

Millet-based mageu 9:1 and millet-based mageu 5:5 were produced following spontaneous fermentation at 25 °C for 4 days and further stored at 4 °C for 2 months. The microbiological properties evaluated were microbial counts for yeasts and moulds, total bacteria, *E. coli* and lactic acid bacteria (LAB). The microbiological analysis included the colony and cellular morphology, biochemical testing, and identification of LAB isolates. The probiotic properties determined were acid and bile salts tolerance while antibiotic susceptibility and antioxidant activity were evaluated for the safety properties of the LAB.

4.3.1. Microbiological evaluation

Day 0 in all observations represented the unfermented gruel following cooking of the cereals and addition of the inoculum, whereas day 4 marked the end of fermentation period for mageu at the specified temperature of 25 °C prior to storage at 4 °C.

4.3.1.1. Enumeration of yeasts and moulds, total bacteria, *E. coli* and LAB of millet-based mageu

Yeasts and moulds, total bacteria and LAB were detected in noticeable amounts while *E. coli* was not detected in millet-based mageu 9:1 and millet-based mageu 5:5.

The yeasts and moulds were detected in both millet mageu types with varying increase in counts over the fermentation and storage period. The rise in total yeast and mould counts was generally non-significant ($p > 0.05$) in both mageu types (Fig 4.1).

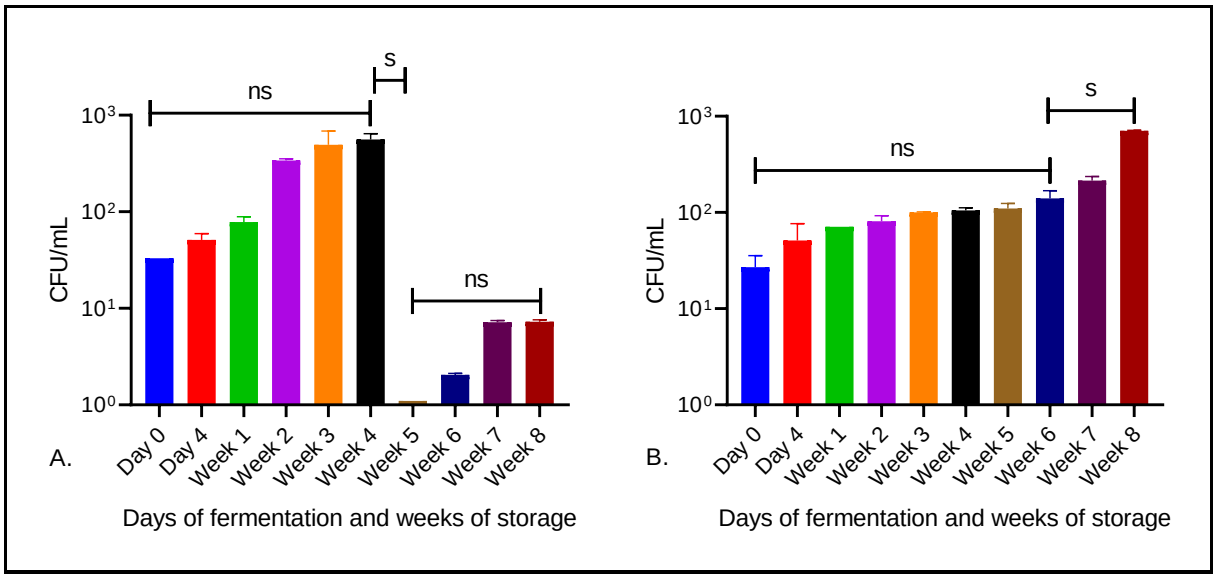

Figure 4 .1: Total yeasts and moulds count in millet-based mageu 9:1 (A) and millet-based mageu 5:5 (B). (S - statistical significance; ns -statistical non-significance).

The fungal count for mageu 5:5 ranged between 2.7×10^1 CFU/mL and 7.05×10^3 CFU/mL from day 0 of fermentation to week 8 of storage while the count in mageu 9:1 ranged between 1.1×10^0 CFU/mL and 5.6×10^3 CFU/mL of fermentation to week 8 of storage. Although the mageu 5:5 had a slightly higher count, both mageu types (mageu 9:1 and mageu 5:5) showed a gradual rise in fungal counts throughout the study period besides the notable significant drop that was observed in mageu 9:1 after week 4.

Total bacterial counts in both millet-based mageu proportions was increasing non-significantly ($p > 0.05$) throughout (Fig 4.2).

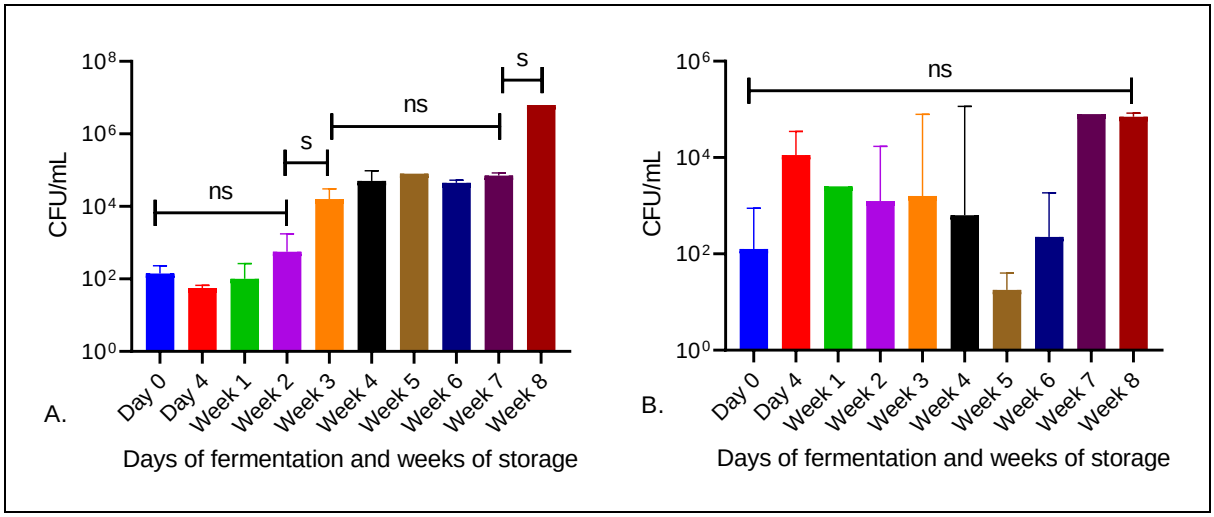

Figure 4.2: Total aerobic bacterial count in millet-based mageu proportions, mageu 9:1 (A) and mageu 5:5 (B). (S - statistical significance; ns - statistical non-significance).

For the millet-based mageu 9:1, the total aerobic bacterial count ranged from $2.0 \times 10^2 – 7 \times 10^5$ CFU/mL although in week 8 the total aerobic bacterial count was higher (7×10^5 CFU/mL). The total bacterial count for millet-based mageu 5:5 ranged from $2.7 \times 10^3 – 1.2 \times 10^5$ CFU/mL with week's 7 – 8 at higher counts (1.2×10^5 CFU/mL). Even though the total bacteria count were increasing among the two proportions, the millet-based mageu 9:1 had the highest count of aerobic bacteria.

The total LAB were higher than total aerobic bacteria, yeasts and moulds in both the proportions of millet-based mageu (mageu 9:1 and mageu 5:5) (Fig 4.3). The total LAB count in millet-based mageu 9:1 and millet-based mageu 5:5 was similar on day 0 of fermentation since LAB was not detected.

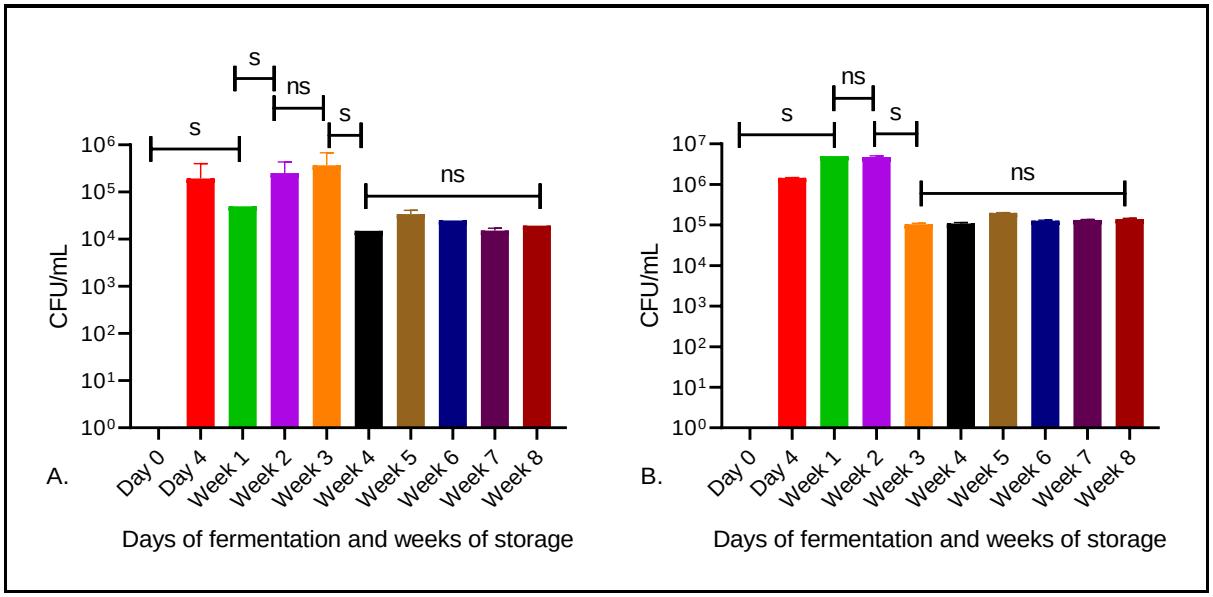

Figure 4.3: Total lactic acid bacterial count in millet-based mageu 9:1 (A) and millet-based mageu 5:5 (B). (S - statistical significance; ns - statistical non-significance).

The LAB count for mageu 9:1 was initially high at 64 × 10^5 CFU/mL and declined to 193 × 10^4 CFU/mL at the end of the study period whilst for mageu 5:5 it declined from 24 × 10^6 CFU/mL to 143 × 10^5 CFU/mL During the first weeks of storage, a gradual increase in LAB count was observed in both the proportions of mageu (mageu 9:1 and mageu 5:5) with a significant decrease ($p < 0.05$) from weeks 3 – 4 of storage for mageu 9:1 and from weeks 2 – 3 for mageu 5:5. The millet-based mageu 5:5 had a greater count of LAB as compared to millet-based mageu 9:1. The significant ($p < 0.05$) difference in the LAB counts between millet-based mageu 9:1 (low count) and millet-based mageu 5:5 (High count) could be attributed to the amount of millet used as inoculum in both mageu products.

4.3.1.2. Lactic acid bacterial isolates

Two different isolates from millet-based mageu 9:1 and millet-based mageu 5:5 were dominant throughout fermentation and storage with consistent characteristics such as a positive Gram stain, shape, margin and elevation (Table 4.2).

Table 4.2: Morphological features of the LAB isolates from millet mageu.

Isolates	**Colony morphology**					**Cellular morphology**	
	Size	**Shape**	**Colour**	**Margin**	**Elevation**	**Gram stain**	**Cell shape**
MD01	Large	Round	Cream white	Entire	Raised	Positive	Coccobacillus
MD02	Small	Round	White	Entire	Raised	Positive	Coccus

Isolate MD01 differed with isolate MD02 with cell shape, colony colour and size.

4.3.1.3. Characterisation and identification of lactic acid bacteria

The isolated LAB isolates of millet-based mageu proportions were found to be similar in all the biochemical tests, even though the identities of the isolates were different (Table 4.3).

Table 4.3: Characteristics and identity of the LAB isolates of millet-based mageu.

Tests			**Isolates**	
			MD01	**MD02**
Catalase test			**-**	**-**
Growth at different salt concentrations	5%	4 °C	**-**	**-**
		25 °C	**+**	**+**
		37 °C	**+**	**+**
	6.5%	4 °C	**-**	**-**
		25 °C	**+**	**+**
		37 °C	**+**	**+**
	13%	4 °C	**-**	**-**
		25 °C	**-**	**-**
		37 °C	**-**	**-**
Growth at different temperatures		4 °C	**-**	**-**
		25 °C	**+**	**+**
		37 °C	**+**	**+**
Motility test			**-**	**-**
Citrate utilisation			**-**	**-**
Sugar fermentation		Lactose	**+**	**+**
		Sucrose	**+**	**+**
		Glucose	**+**	**+**
Nitrate reduction			**+**	**+**
Oxidase test			**+**	**+**
Blood agar test			**-**	**-**
Identities of the isolates (by molecular approach)			***Pediococcus pentosaceus***	***Pediococcus acidilacti***

Key: The + sign denotes a positive result and - sign denotes a negative result.

Isolate MD01 was identified as *Pediococcus pentosaceus* while isolate MD02 was identified as *Pediococcus acidilacti*. Both isolates were present throughout the fermentation process and the period of storage.

4.3.2. Acid tolerance of the *Pediococcus* strains

Pediococcus pentosaceus MD01 and *P. acidilacti* MD02 exhibited a high survival rate at different acidic conditions (Fig 4.4). During the 36 hours incubation period, a significant ($p < 0.05$) difference in the acidic conditions was noted.

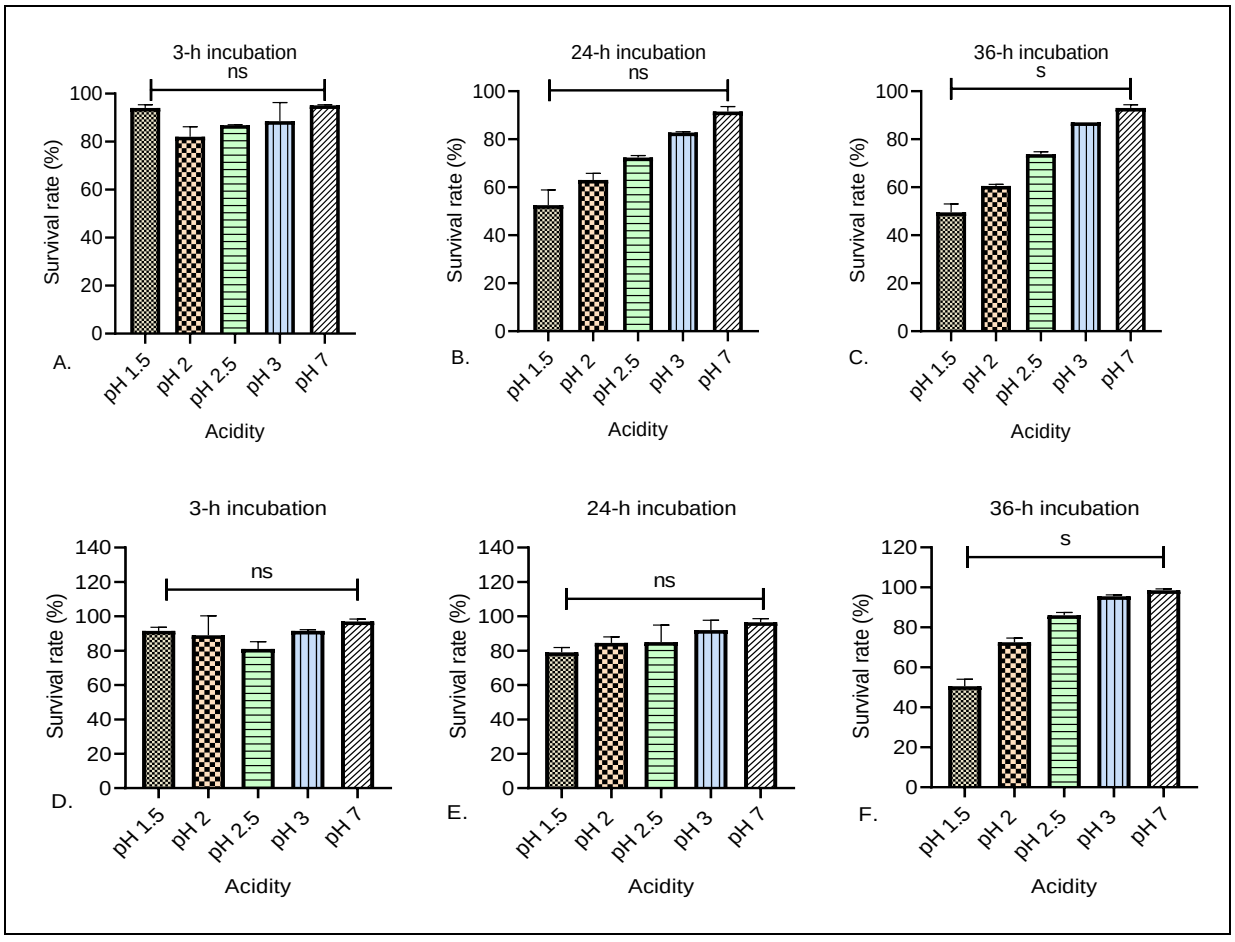

Figure 4.4: Survival rate of *P. pentosaceus* MD01 at 3 hours (A), 24 hours (B) and 36 hours (C) and *P. acidilacti* MD02 at 3 hours (D), 24 hours (E) and 36 hours (F) under acidic conditions. (S - statistical significance; ns - statistical non-significance; h - hour).

The survival rate of *P. pentosaceus* MD01 and *P. acidilacti* MD02 at pH 1.5, 2, 2.5, 3 and 7 was higher within a range of 80% to 90% after three hours of incubation. Both the LAB showed sensitivity to pH 1.5 when exposed for a longer period whereas

moderate acidity of pH 3 did not affect *P. pentosaceus* MD01 and *P. acidilacti* MD02 even under an extended period of exposure.

4.3.3. Bile salts tolerance

P. pentosaceus MD01 was sensitive to a long period of exposure to low concentrations of salt (at a moderate pH of 3) whereas *P. acidilacti* MD02 showed sensitivity to a moderate pH 3 when exposed for a short period of time (Fig. 4.5). The non-significant difference ($p > 0.05$) was noted in 0.3% and 0.9% bile salt at 24 and 48 hours of incubation.

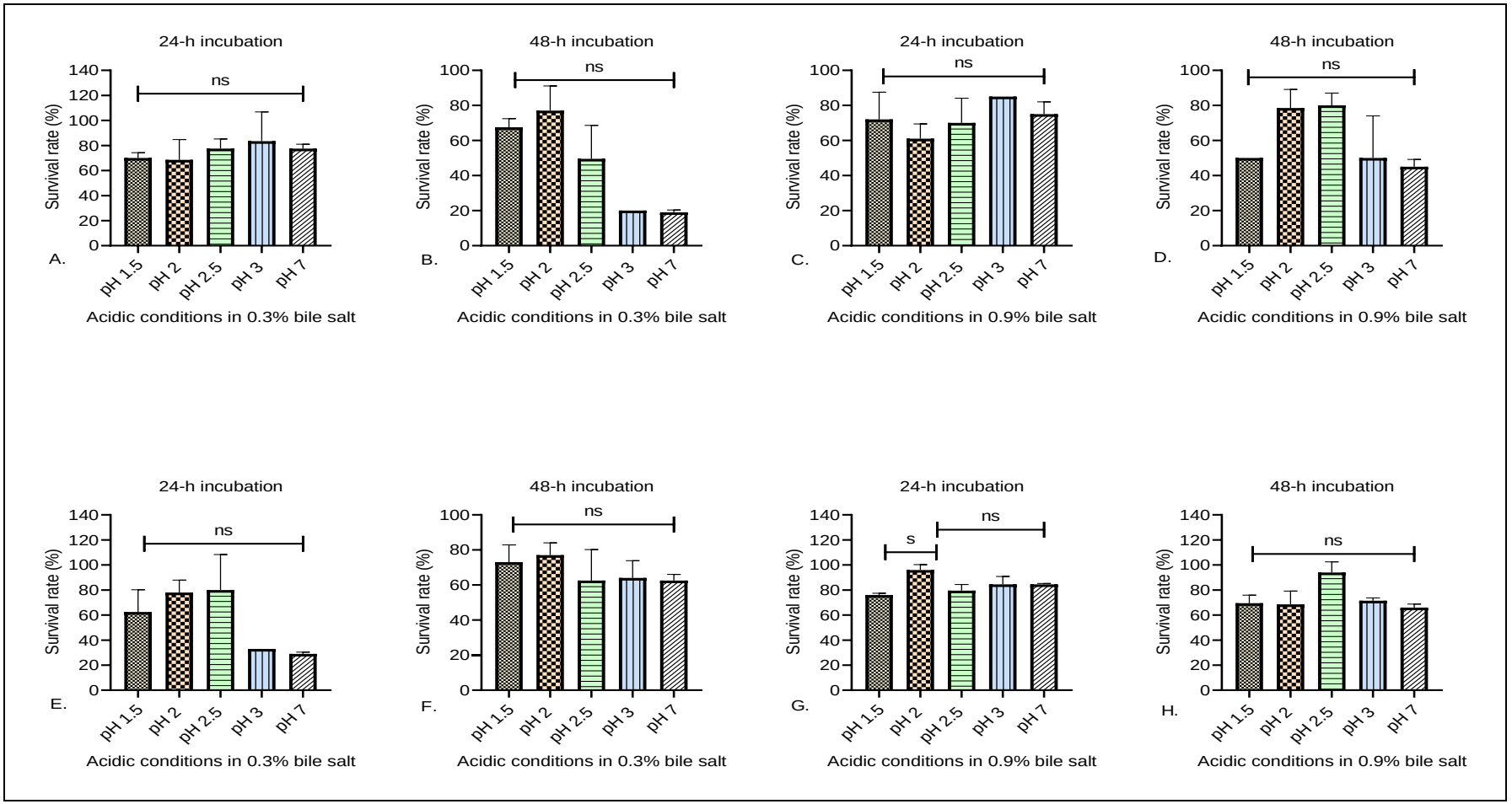

Figure 4.5: Survival rate of *P. pentosaceus* MD01 after 24 hours (A) and 48 hours (B) of incubation at different acidic conditions in MRS supplemented with 0.3% bile salt and, 24 hours (C) and 48 hours (D) incubation in 0.9% bile salt. Survival rate of *P. acidilacti* MD02 after 24 hours (E) and 48 hours (F) of incubation at different acidic conditions in MRS supplemented with 0.3% bile salt and, 24 hours (G) and 48 hours (H) incubation in 0.9% bile salt. (NS - statistical non-significance; h - hour)

Both isolates showed a good tolerance to bile salt conditions. At lower bile salt concentrations, *P. pentosaceus* showed tolerance in all the acidic conditions when exposed for shorter incubation time while *P. acidilacti* was able to tolerate exposure for longer periods of incubation in all the acidic conditions. However, both isolates survived very well at higher salt concentration for both shorter and extended period of exposure. Interestingly, a drop in the survival rate of *P. pentosaceus* was noted after 24 hours of incubation at 0.3% bile salt and 0.9% bile salt after 48 hours of incubation especially for the *P. pentosaceus* isolate that survived on the moderate acidic environment of pH 3 whereas for *P. acidilacti* the opposite was noted. An increase in the survival rate of *P. acidilacti* isolate (at pH 3) was noted at 0.3% of bile salt after shorter exposure (24 hours) with a slight drop at 48 hours of incubation for 0.9% bile salt.

4.3.4. Antibiotic susceptibility test

P. pentosaceus MD01 and *P. acidilacti* MD02 were resistant to penicillin and streptomycin, but susceptible to chloramphenicol and displayed contrasting sensitivity to tetracycline (Table 4.4).

Table 4.4: Antibiotic susceptibility profile of *P. pentosaceus* MD01 and *P. acidilacti* MD02.

Antibiotics (dosage)	**Lactic acid bacteria isolates**			
	***P. pentosaceus* MD01**		***P. acidilacti* MD02**	
	Zone size (mm)	**Susceptibility**	**Zone size (mm)**	**Susceptibility**
Penicillin (10 µg)	30.0 ± 0.07	Resistant	20.2 ± 0.28	Resistant
Streptomycin (10 µg)	0.9 ± 0	Resistant	0.95 ± 0.07	Resistant
Tetracycline (30 µg)	30.1 ± 0.14	Susceptible	10.9 ± 0	Resistant
Vancomycin (30 µg)	10.65 ± 0.07	Intermediate	10.85 ± 0.21	Intermediate
Chloramphenicol (30 µg)	20.45 ± 0.21	Susceptible	20.35 ± 0.07	Susceptible

The ± indicate the standard deviation of the zone's sizes.

4.3.5. Antioxidant activity of the isolates

The two LAB strains exhibited an intermediate level of antioxidant activity that were similar after 24 hours of incubation at 34% and increased non-significantly after a further 24 hours incubation (Table 4.5). The non-significant ($p > 0.05$) difference in the scavenging activity of *P. pentosaceus* MD01 and *P. acidilacti* MD02 after 24 and 48 hours of incubation was observed.

Table 4.5: Antioxidant activity of the isolated lactic acid bacteria during growth in MRS broth.

Strains	**DPPH radical scavenging activity (% Inhibition)**	
	24 hours	**48 hours**
P. pentosaceus MD01	34.26 ± 0.013	38.85 ± 0.002
P. acidilacti MD02	34.14 ± 0.002	35.56 ± 0.180
L-ascorbic acid	80.56 ± 1.13	78.60 ± 0.800

4.4. Discussion

The current chapter outlines the microbiological content of mageu produced from millet grains with different proportions of millet flour. The chapter further outlines the probiotic, health and safety properties of lactic acid bacteria (LAB) isolated from the millet-based mageu products. The production of mageu followed the natural fermentation process with millet flour used as the inoculum to initiate fermentation. It is recognised that the microorganisms detected in both proportions of millet-based mageu could have resulted from millet flour, maize meal, water and utensils that were used during the preparation of mageu (Hassanzadazar *et al*., 2012; Mashau *et al*., 2020) although the utensils where sterilised before use.

The yeasts and moulds, total bacteria and lactic acid bacteria were observed throughout the fermentation and storage periods. It is appreciable that *Escherichia coli* was not detected during fermentation and storage of the mageu products. *E. coli* is regarded as an indicator organism for health risk in food and water (Ekici and Dumen, 2019), hence its absence in the millet-based mageu products implies that good hygiene was achieved (Mashau *et al*., 2020).

The total bacterial count in millet-based mageu was fluctuating throughout and this could be due to the acidic environment created by LAB as some of the bacteria cannot survive in acidic conditions (Laranjo *et al*., 2019). Moreover, LAB have the bioprotective effect of other microorganisms thereby controlling their growth by producing antagonistic compounds (enzymes and hydrogen peroxide) and bacteriocins (Laranjo *et al*., 2019). Food products are expected to have a low bacterial count as this indicates good quality of the food product as well as acceptable conditions of the ingredients (or raw materials) used (Abakari *et al*., 2018). This should be below 10^3 CFU/mL according to the Food and Drug Administration (FDA) (Leone, 2014). The unfermented gruel (Day 0) had low total bacterial count ranging from 2.0×10^2 CFU/mL – 2.7×10^3 CFU/mL and therefore complies with the limits set by the FDA. As the fermentative conditions started, the aerobic bacterial counts increased.

Lactic acid bacteria are Gram-positive and mostly rod shaped (Bintsis, 2018). The LAB strains isolated in this study displayed biochemical characteristics that are congruent with known properties of lactic acid bacteria. LAB can ferment sugars, grow under different salt concentrations, have broader growth temperatures range, can reduce nitrate and have the ability to oxidase cytochrome C (Ismail *et al*., 2018).

LAB were not detectable in the unfermented gruel of millet-based mageu. Other studies reported low numbers of LAB, in the range of 3.00 – 5.45 CFU/mL, although this was in maize-based mageu (Mashao *et al*., 2020). The growth of LAB strains in fermentation processes is due to the presence of an acidic environment and some specific nutrients (Simatende *et al*., 2019). Yeasts reportedly initiate fermentation thereby providing nutrients and growth factors (such as vitamins) to enhance the growth of LAB (Ponomarova *et al*., 2017). The limitation of such nutrients could contribute to the drop in LAB counts during storage when yeasts count decrease as well. However, the dominance of LAB in both the proportions of millet-based mageu highlight that the LAB are the main drivers of fermentation in this study, similar to a report on food fermentation made elsewhere (Oh and Jung, 2015). Furthermore, this dominance might be resulting from the ability of LAB to restrain the growth of other microorganisms due to competition for resources (Mashau *et al*., 2020). Notably, the organic acids and deacetyl's produced by LAB are known to inhibit the development of yeasts and moulds (Mashau *et al*., 2020), and this could explain the low fungal

counts in both mageu products throughout fermentation. The presence of fungi in high numbers in food products can be a concern due to mycotoxins that some fungal species produce, which adversely affect the health of humans (Adisa *et al*., 2020).

Most LAB strains are beneficial as probiotics that are good for the gut of humans (Bonatsou *et al*., 2017). The ability of *Pediococcus acidilacti* MD02 and *Pediococcus pentosaceus* MD01 to survive well in conditions of varying osmotic pressure and different temperature ranges (25 °C and 37 °C) could be beneficial for metabolism as probiotics (Maakelo *et al*., 2021*). Pediococcus* species are not commonly isolated from fermented foods as compared to *Lactobacillus* species, which were reported mainly in maize meal-based mageu (Idowu *et al*., 2016; Salvado *et al*., 2016). Probiotics are essentially good bacteria that benefit individuals when consumed in sufficient amounts (Bintsis, 2018), by keeping a microbial balance in the stomach and intestinal tract (Oh and Jung, 2015). The identified LAB isolates, *P. pentosaceus* MD01 and *P. acidilacti* MD02 were able to withstand low pH of simulated acidic conditions of the stomach at a native state, and during and after digestion of a meal. The survival of these isolates in these acidic conditions depends on their intrinsic tolerance as well as the food matrix harbouring them (Garcia *et al*., 2016). Their survival rate for extended times (24 and 26 hours) at a low pH of 2.5 and 3 is a good probiotic attribute since the stomach pH rises from the resting acidic state of pH 1 – 2 (Amara *et al*., 2019) to pH 3 – 5 (Koziolek *et al*., 2015) during food digestion, depending on the type of food consumed (Dressman *et al*., 1990). LAB are organisms that are generally regarded as safe (GRAS) hence they can be used safely in medical application to improve the health of humans (Anitha *et al*., 2018). The important characteristic of LAB strains to survive and often thrive in the intestinal tract, is their resistance to the bile secretions (Oh and Jung, 2015). The identified isolates displayed the ability to survive and tolerate the bile conditions of the stomach. *P. pentosaceus* MD01 and *P. acidilacti* MD02 were able to resist the bile conditions at 0.3% concentration and 0.9% concentration (critical concentration) after a 24 hours period. The tolerance to bile conditions by these strains is significant for creating a healthy equilibrium between the beneficial and potentially harmful microflora thereby preventing intestinal infection (Shehata *et al*., 2016).

Survival of probiotics under harsh conditions is key to their beneficial role in the human gut. *Pediococcus pentosaceus* MD01 and *P. acidilacti* MD02 exhibited resistance to

antibiotics such as penicillin and streptomycin, inferring that these strains will continue to grow in the gut of individuals under antibiotic treatment (Reller *et al*., 2009). Other researchers (Anitha *et al*., 2018; Oh and Jung, 2015) demonstrated the resistance of *P. acidilacti* and *P. pentosaceus* to antibiotics, and they are considered naturally resistant to several antibiotics. The resistance of LAB species to streptomycin is intrinsic because their resistance is encoded in the chromosomes (Anitha *et al*., 2018). Both strains showed susceptibility to chloramphenicol and intermediate susceptibility to vancomycin. Interestingly, the two strains exhibited contrasting reactions to tetracycline, with *P. acidilacti* MD02 displaying resistance whereas *P. pentosaceus* MD01 being susceptible to the antibiotic. In South African health facilities, the antibiotics such as streptomycin, vancomycin, tetracycline, and penicillin are used as the first line of defence when treating bacterial infections, with penicillin being used mostly as first line of protection for bacterial infections and other health related infections such as pharyngitis (Jahantigh *et al*., 2020).

Antioxidants are known for their significance in preventing cell damage (Chong *et al*., 2023). A diet containing plenty of vegetables, fruits and grains (fermented or unfermented) is considered rich in antioxidants (Lin *et al*., 2009). There is no recommended limit to the amount of antioxidants an individual can consume. However, health risks such as prostate cancer, lung cancer and stroke have been linked to high doses of antioxidant supplements such as vitamin E and beta-carotene (Lin *et al*., 2009). *P. pentosaceus* MD01 and *P. acidilacti* MD02 were observed to possess moderate antioxidant activity of 34.14% – 38.85% for short (24 hours) and longer (48 hours) incubation hours. Oh and Jung (2015) observed the same level of antioxidant activity for these strains during production of omegisool, which is a traditionally fermented millet alcoholic beverage. The antioxidants are beneficial towards improving the innate immunity of the intestinal tract of individuals (Chong *et al*., 2023). The antioxidant activity of *P. acidilacti* MD02 and *P. pentosaceus* MD01 could contribute to the improvement of mucosal proliferation and act as a barrier function in human beings after exposure to certain diseases or neurological disorders (Wang *et al*., 2021).

The presence of yeasts played a significant role in lowering pH as well as supplying nutrients to support the growth of LAB that are recognised as probiotics (Mashau *et*

al., 2020; Ponomarova *et al*., 2017). The survival and tolerance of the LAB isolates, *P. pentosaceus* MD01 and *P. acidilacti* MD02 to the acidic environment and bile salts, their ability to produce antioxidants as well as being resistant towards certain antibiotics are significant towards the health of individuals. Regular inclusion of millet-based mageu in human diet could consequently protect against microbial infections as well as boosting the immune system (Anitha *et al*., 2018) based on these good health properties.

CHAPTER 5. EVALUATION OF THE CHEMICAL AND NUTRITIONAL PROPERTIES OF A MILLET-BASED MAGEU

5.1. Introduction

Mageu is a non-alcoholic beverage that is mostly consumed as an anytime meal in South Africa by all age groups (Idowu *et al*., 2016). Mageu is known for its sour taste, which is influenced by the presence of acids and low pH (Eswatini, 2019). It is commonly produced in summer due to the warm weather that favours the fermentation of mageu (Eswatini, 2019). The production of mageu follows the natural fermentation process and maize flour, leftover maize porridge as well as sorghum flour or the combination thereof are commonly used (Idowu *et al*., 2016).

The chemical composition and quality of mageu is influenced by the raw materials used, microbial activities during fermentation and storage conditions of the product after fermentation (Khan *et al*., 2022). Microorganisms play a major role in the production of various organic acids; especially the lactic acid bacteria (LAB) that produce lactic acid. LAB dominate in mageu fermentation (Mashau *et al*., 2020), hence most studies attribute the drop in pH and acidification due to the production of lactic acid and other organic acids (Maakelo *et al.,* 2021). The pH of most mageu products ranges from pH 3 – 4.5 with acidity between 0.05% – 0.6% (Mashau *et al*., 2020). Such pH and total titratable acidity (TTA) levels are beneficial towards inhibition of many pathogens (Maakelo *et al.,* 2021).

The activity of the dominating microorganisms and the type of grains used both influence sugar content in mageu. The total soluble sugar in mageu increases during fermentation and storage of mageu (Mashau *et al*., 2020) due to saccharification of complex carbohydrates in the substrates such as maize and sorghum. These simple sugars are metabolised to lactate (Maakelo *et al.,* 2021) and other organic acids.

The nutritional value of mageu is associated with increased levels of moisture content ranging from 80 – 95% (Maakelo *et al*., 2021), vitamins B and C and different minerals (Mashau *et al*., 2020). Mageu is reported to contain high levels of inorganic mineral

elements (ash) with the content between 0.36 – 2.27% (Boyiako *et al*., 2020). The level of proteins in mageu tends to be unappreciable due to lower levels of essential amino acids such as lysine and the coarse nature of maize and sorghum grains (Fadahunsi and Soremekun, 2017). Additionally, mageu is deficient in other nutrients such as carbohydrates, copper, zinc, iron, and fats (Fadahunsi and Soremekun, 2017). While these nutrients are important for the overall quality of mageu, they are mostly lacking in other types of fermented cereal grains (Fadahunsi and Soremekun, 2017).

Besides factors such as acidity, pH, sugar, antioxidant activity and microbial load, the type of grain used also contributes towards a better nutritional and chemical profile of mageu. Most antioxidants are extracted from the raw materials, especially from the bran and germ of the cereal grains (Khan *et al*., 2022). Grains such as millets have a unique nutritional profile and are good sources of energy (Adebiyi *et al*., 2018). Millet has a higher fat content, dietary fiber and essential amino acids compared to maize meal (Khan *et al*., 2022). Millet is used in an unrefined state and therefore possesses higher amounts of bioactive compounds such as flavonoids, tannins, phenolics, terpenoids and polyphenols (Nithiyanantham *et al*., 2019) as well as vitamins and minerals, particularly phosphorus, potassium, calcium, sodium, and magnesium (Johnson *et al*., 2019).

Millet has not been widely explored in the production of mageu. This could be attributed to its naturally bitter taste. The current chapter reports on the characteristics of mageu produced from fermentation of pearl millet in terms of the chemical composition and the nutritional content.

5.2. Materials and methods

5.2.1. Preparations of millet-based mageu

Two proportions of millet-based mageu were prepared with a total volume of 7 L following a modified method of Fadahunsi and Soremekun (2017). The proportions were prepared as outlined in chapter 4, section 4.2.1.

5.2.2. Chemical characterisation of mageu

5.2.2.1. pH

The pH was measured as described in chapter 3, section 3.2.2.1.

5.2.2.2. Total titratable acidity

Total titratable acidity (TTA) was determined as outlined in chapter 3, section 3.2.2.2.

5.2.2.3. Determination of the antioxidant activity of mageu

Antioxidant activity of the millet-based mageu was determined following the method of Chigayo *et al*. (2016). The samples of the fermented brew were dried under a fan using cold air at room temperature and afterwards ground into a fine powder using a blender. Methanol, acetone, and hexane were used for extraction. One gram of the dried samples was transferred into a 50 mL centrifuge tube followed by addition of 10 mL of the extraction solvent. The mixture was incubated in a shaking incubator (New Brunswick Scientific) for 10 minutes at 200 rpm at 37 °C. The extracts were then filtered using Whatman No.1 (22 mm diameter) filter papers into the vials that were pre-weighed and labelled accordingly. The vials containing the extracts were then dried using cold air at room temperature. Following drying, the weight of the vials was measured and recorded. All the extracts were reconstituted in acetone to a final volume of 10 mg/mL.

Free radical scavenging activity was determined using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) that was prepared using methanol (Chigayo *et al*., 2016). A L-ascorbic acid standard was prepared in triplicates in the concentration range of 250 – 15.63 µg/mL using 2 mL of a DPPH solution. The L-ascorbic acid was covered with aluminium foil due to its light sensitivity. Each 1 mL extract was prepared with 0.5 mL of the acetone extract and 0.5 mL of acetone. Thereafter, 2 mL DPPH was added to the extract solution. The control was prepared by mixing 1 mL acetone and 2 mL DPPH whereas the blank was prepared using 1 mL acetone and 2 mL methanol. The solutions were also covered with aluminium foil. The solutions were mixed thoroughly by vortex and incubated in the dark for 30 minutes, then mixed again thoroughly. The absorbance

readings of L-ascorbic acid, control, blank and the extracts was measured using an ultraviolet spectrophotometer (Thermo Scientific) at a wavelength of 517 nm. The effective concentration (EC_{50}%) was calculated from the standard curve. Thereafter, the effective concentration (EC_{50}%) percentage was expressed according to the formula below which is represented as the DPPH radical scavenging activity (EC_{50}) of all the extracts and L-ascorbic acid.

$$Effective\ inhibition\ (\%) = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control}\ X\ 100$$

5.2.3. Determination of nutritional content of mageu

5.2.3.1. Protein content

The fermented brew was dried under a flow of cold air at room temperature. The salt/alkaline extraction method was used to extract proteins from the mageu samples and performed in triplicates (Mæhre *et al*., 2018). Zero-point five gram (0.5 g) of the dried mageu samples was transferred into 50 mL centrifuge tubes followed by the addition of 30 mL 0.1M NaOH in 3.5% NaCl. The mixture was homogenised using ultrasonication for 40 seconds and incubated at 60 °C for 90 minutes. After incubation, the homogenate was further centrifuged for 30 minutes at 4000 x g at 4 °C to obtain the protein extracts. The protein extracts were assessed using the QuantiPro™ Bicinchoninic Acid Assay (BCA) assay kit (Sigma-Aldrich) as indicated by the protocol of the manufacturer. Bovine Serum Albumin (BSA) was used as the standard and the protein content was determined by extrapolating from the BSA standard and the results were expressed in µg/mL.

5.2.3.2. Fat content

The fat content of the mageu samples was extracted in triplicates using the chloroform: methanol (1:2 v/v) method outlined by Folch *et al*. (1957) with minor modifications that were obtained from Holman *et al*. (2019). Three millilitres (3 mL) of the mageu samples were transferred into 50 mL centrifuge tubes using Pasteur pipettes. The sample was mixed with 3.75 mL chloroform and 7.5 mL methanol. The mixture was incubated in a shaking incubator at 200 rpm or 1 hour at 25 °C. Thereafter 3.75 mL chloroform and

distilled water (3.75 mL) were added to the sample mixture. The top layer was removed using glass Pasteur pipettes and discarded. Three point six eight millilitres (3.68 mL) of chloroform: methanol: distilled water (3:48:47 v/v) was added and used to wash the bottom layer. The mixture was left at room temperature for 5 minutes. The top layer was removed again. The washing procedure was repeated. The remaining bottom layer which contained fats was transferred into the pre-weighed clean vials. The vials containing the fat extract were then evaporated at room temperature under a stream of cold air. After drying, the vials were weighed, and the mass was recorded. The crude fat percentage was expressed according to the following formula and was reported in g/mL.

$$Crude\ fat\ (\%) = \frac{(Weight\ of\ vial\ with\ dried\ fat - Weight\ of\ vial)\ X\ 100}{Sample\ in\ mL}$$

5.2.3.3. Moisture and Ash content

The moisture content and total mineral content were determined using the ash method (AOAC, 2005). The crucibles were cleaned thoroughly. Pasteur pipettes were used to transfer 5 mL of the mageu sample into the cleaned crucibles. The crucibles containing the sample were weighed and recorded. The crucibles were then taken into an oven and allowed to dry for 24 hours at 70 °C. After drying, the crucibles were removed from the oven. The weight of the dried sample in the crucible was measured and recorded in triplicates. The percentage moisture content was then calculated using the following formula:

$$\%\ Moisture = \frac{Weight\ of\ crucible - weight\ of\ crucible\ with\ dried\ sample}{Weight\ of\ crucible - weight\ of\ crucible\ with\ wet\ sample}\ X\ 100$$

Following determination of moisture content, the crucibles containing the dried mageu samples were transferred into the furnace and burned to a white powder at 600 °C for 24 hours. The crucibles were then removed from the furnace and transferred into a desiccator and allowed to cool for 30 minutes. The mass of the crucibles containing the burnt samples were determined. The percentage of ash was calculated according to the formula below.

$$\%Ash = \frac{Weight\ of\ the\ ash}{Weight\ of\ the\ original\ sample}\ X\ 100$$

5.2.3.4. Mineral composition

The mineral content for millet-based mageu proportions was performed at LATS (University of Limpopo). The mageu samples were first dried in an oven at 25 °C for 24 hours to evaporate the water. Sample digestion was done in a digestion vessel whereby 400 mg of each dried sample was mixed with 5 mL of nitric acid (HNO_3) and 2 mL of hydrogen peroxide (H_2O_2). The mixture was mixed carefully and left at room temperature for 10 minutes. The vessel was then closed and heated in the microwave at 170 °C and 30 bar (pressure) for 10 minutes with 80% power at 15 minutes as the holding time. The vessels were then cooled for 20 minutes at 25 °C. The digestion vessels were opened carefully in the fume hood and the samples were analysed for the presence of the following minerals: calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) using the Shimadzu Inductively Coupled Plasma Emission Spectroscope in triplicates (ICPE-9000) (Poitevin, 2012). The mineral contents were expressed in mg/mL.

5.2.3.5. Sugar content

The mageu samples were filtered into 2 mL vials using a syringe and sterile 0.45 µm filters. The sample volume of 1.5 mL (in triplicates) was used to detect and measure the concentrations of sucrose, glucose and fructose using High-Performance Liquid Chromatography (HPLC) (Ramoba *et al*., 2022). The standard curves were prepared using standard analytical sugars for sucrose, glucose, and fructose within the concentration range of 0.390 – 400 mg/mL. Deionised water was used as the mobile phase and a Rezex RHM monosaccharide column was used and operated at 80 °C at a flow rate of 0.600 mL per minute. The separated components in the samples were detected using a refractive index detector. The concentration of the samples was then calculated using the standard curves and the sugars were recorded in mg/mL.

5.2.4. Statistical analysis

The results were analysed using one-way ANOVA followed by Tukey's multiple comparisons of the graph pad prism 8. Excel was used to measure the standard deviation of the samples. Significant differences were determined at $p \leq 0.05$.

5.3. Results

The two proportions of millet-based mageu (9:1 and 5:5) were produced through natural fermentation at ambient temperature (25 °C) for four days and thereafter stored for 8 weeks at 4 °C. The chemical characteristics that were determined included pH, TTA and antioxidants activity as well as the nutritional composition including protein content, crude fats, ash and moisture content, mineral composition, and sugars.

5.3.1. Chemical parameters of millet-based mageu

5.3.1.1. pH

A significant ($p < 0.05$) decrease in pH was observed during fermentation, from day 0 to day 4 in the two mageu preparations (Fig. 5.1), whereas it remained constant during storage. The pH remained within a range of 3.21 – 3.55 during storage for the millet-based mageu 9:1 (Fig. 5.1A) and it was higher at a pH range of 4.0 – 4.16 for the millet-based mageu 5:5 (Fig. 5.1B).

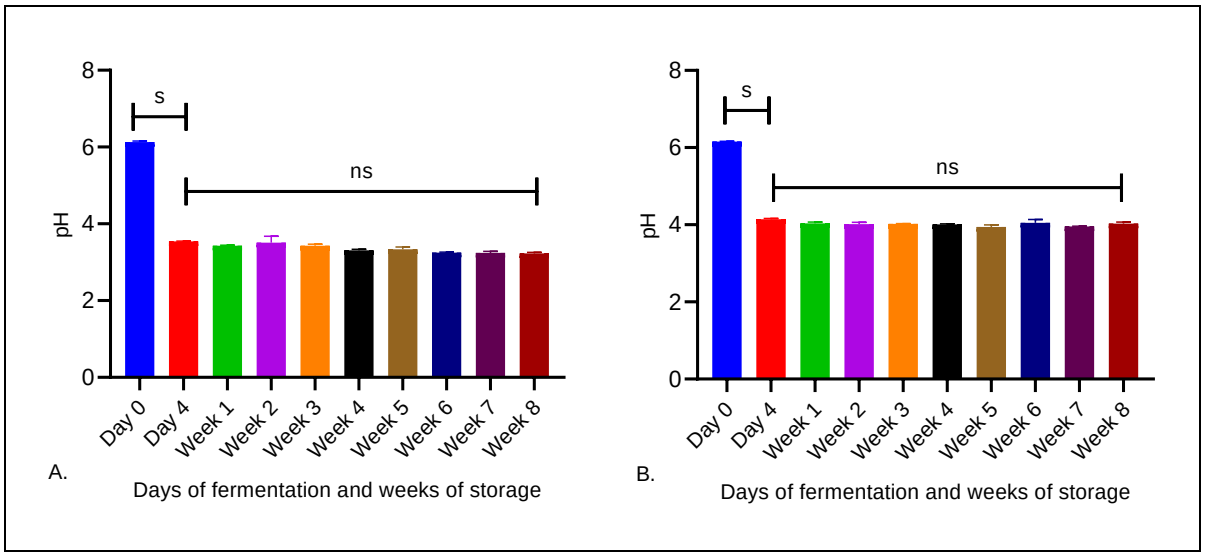

Figure 5.1: pH profiles of millet-based mageu 9:1 (A) and millet-based mageu 5:5 (B). S denotes statistical significance and ns statistical non-significance.

5.3.1.2. Total titratable acidity

Total titratable acidity was used to check the total amount of organic acids in the millet-based mageu products and the %TTA profile of both the proportions is indicated in figure 5.2.

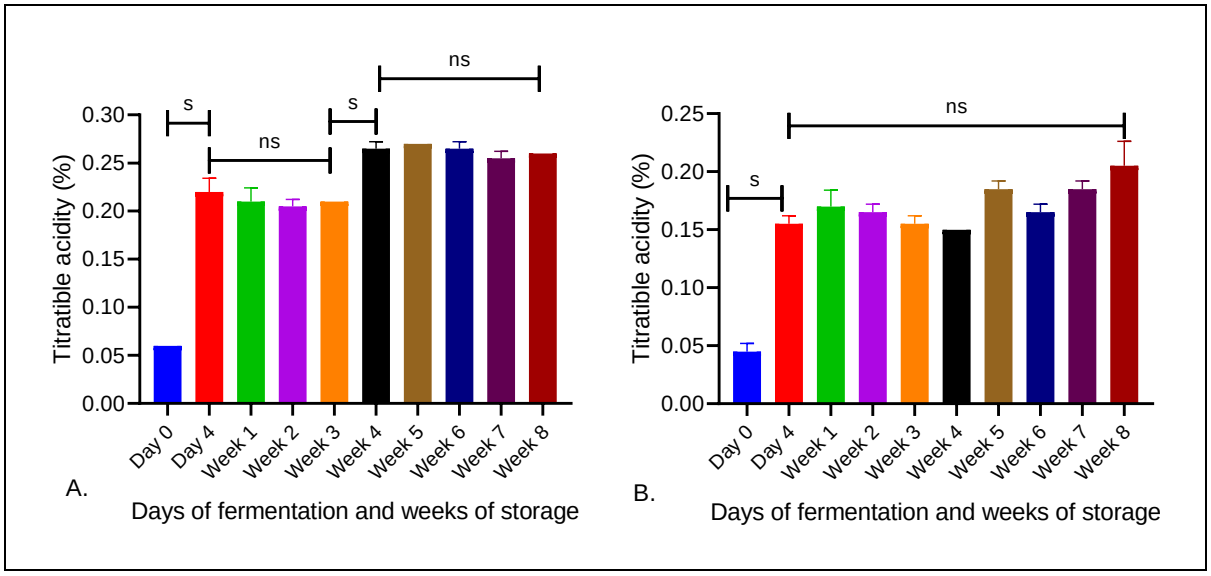

Figure 5.2: TTA of mageu proportions, A is 9:1 proportion and B is 5:5 proportion. S denotes statistical significance and ns is statistical non-significance.

The proportions showed a significant ($p < 0.05$) increase in total titratable acidity from day 0 to day 4 of fermentation. The sharp increase in total acidity on the first day of fermentation for mageu preparations (Figure 5.2) corresponded to the steep decline of pH as depicted in Fig. 5.1. Similarly, total acidity remained relatively constant during storage for both proportions at the ranges of 0.16 – 0.22% for millet-based mageu 5:5 (Fig. 5.2 B) and 0.23 – 0.27% for millet-based mageu 9:1 (Fig. 5.2A). The millet-based mageu 9:1 produced comparatively more organic acids and a lower acidic pH.

5.3.1.3. Antioxidant activity

Figure 6.3 represents the antioxidant activities of millet-based mageu preparations. L-ascorbic acid was used as the standard and the results were expressed as the DPPH radical scavenging activity (EC_{50}) concentrations.

There is a general non-significant fluctuation in antioxidant activities among the different extracts in both mageu preparations during storage (Fig. 5.3). Generally, all

the extracts showed lower antioxidant activity, although methanol and hexane notably extracted significantly ($p < 0.05$) higher antioxidants as compared to the acetone extracts in both mageu preparations.

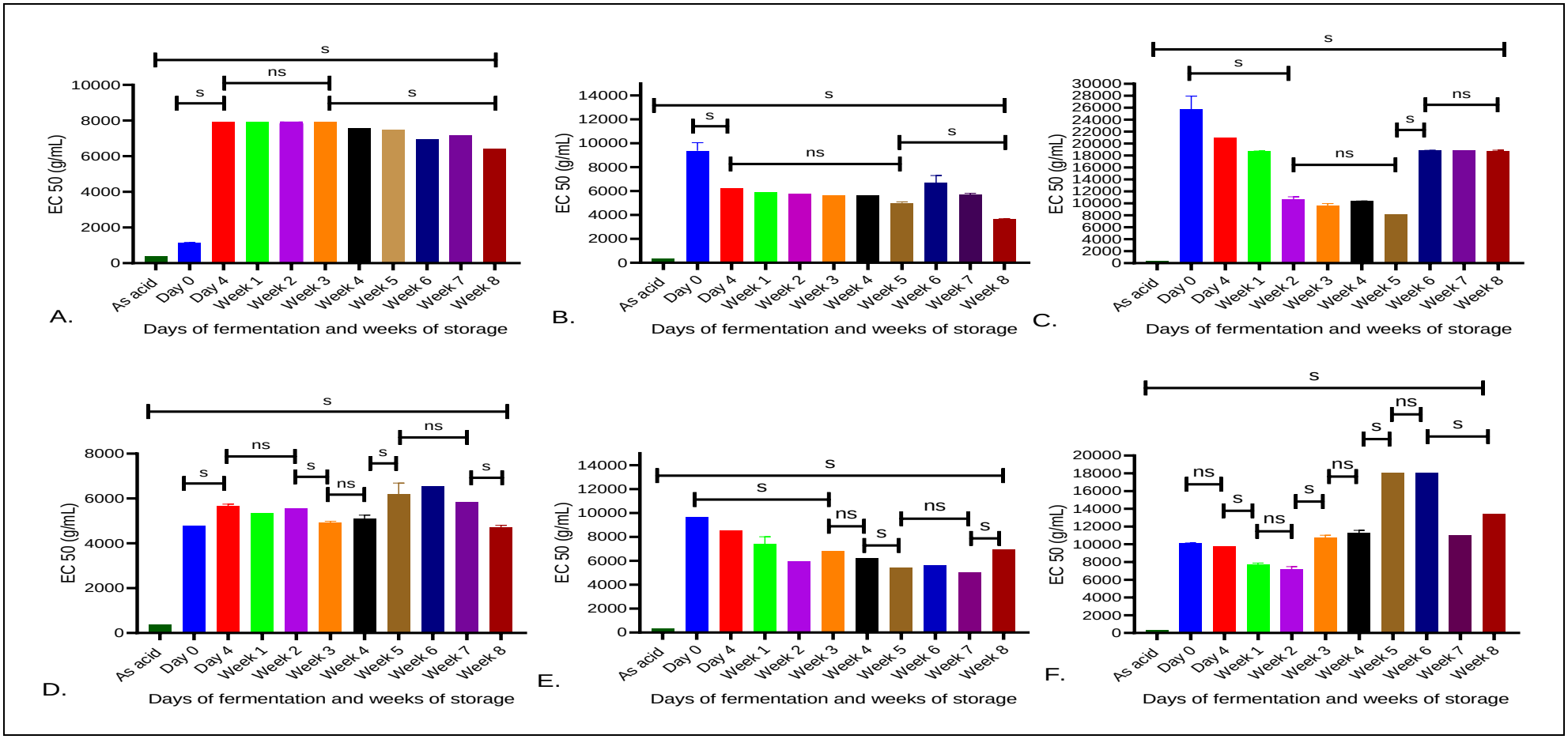

Figure 5.3: Antioxidant activity of the millet-based mageu preparations. A – C represent methanol, hexane, and acetone extracts of millet-based mageu 9:1, respectively; D – F represent methanol, hexane, and acetone extracts of millet-based mageu 5:5, respectively. S denotes statistical significance; ns is statistical non-significance and As acid indicates Ascorbic acid.

5.3.2. Nutritional composition

5.3.2.1. Protein content

The protein content of mageu is indicated in Fig 5.4 and it was expressed in µg/mL.

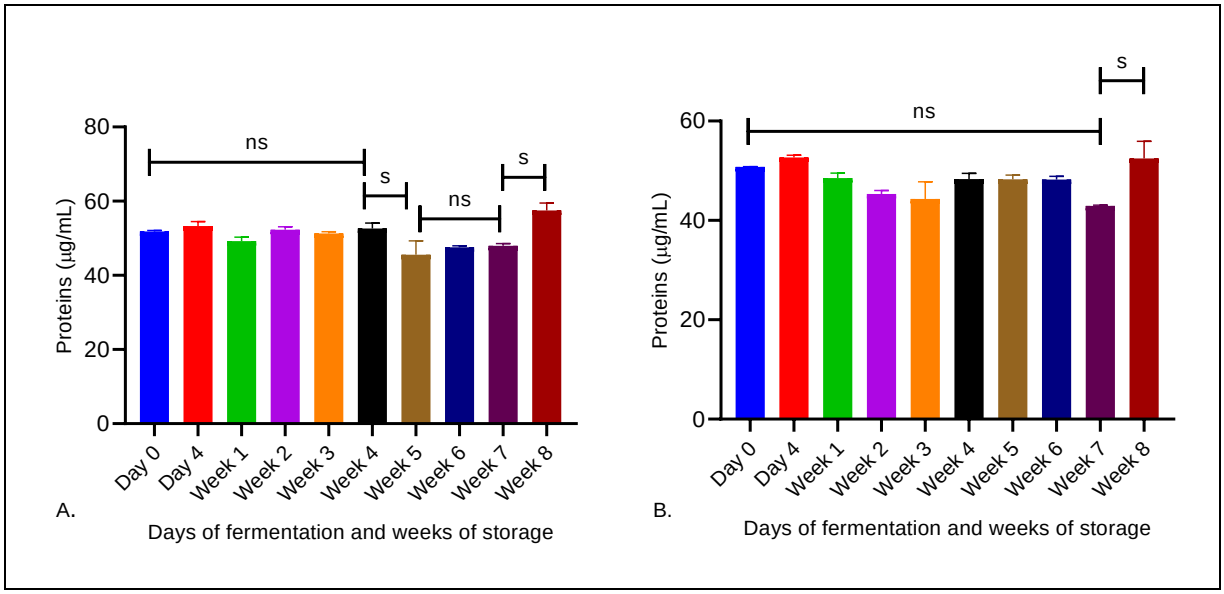

Figure 5.4: Protein content of mageu 9:1 proportion (A) and protein content of mageu 5:5 proportion (B). S denotes statistical significance and ns statistical non-significance.

There is generally a non-significant fluctuation ($p > 0.05$) in protein content in both mageu preparations. The protein content ranged between 42 – 58 µg/mL for both the mageu 9:1 (Fig. 5.4A) and mageu 5:5 (Fig. 5.4B) and there was no difference observed between fermentation and storage phases.

5.3.2.2. Crude fat content

Crude fat content of mageu was expressed in g/mL and is noted in Fig. 5.5.

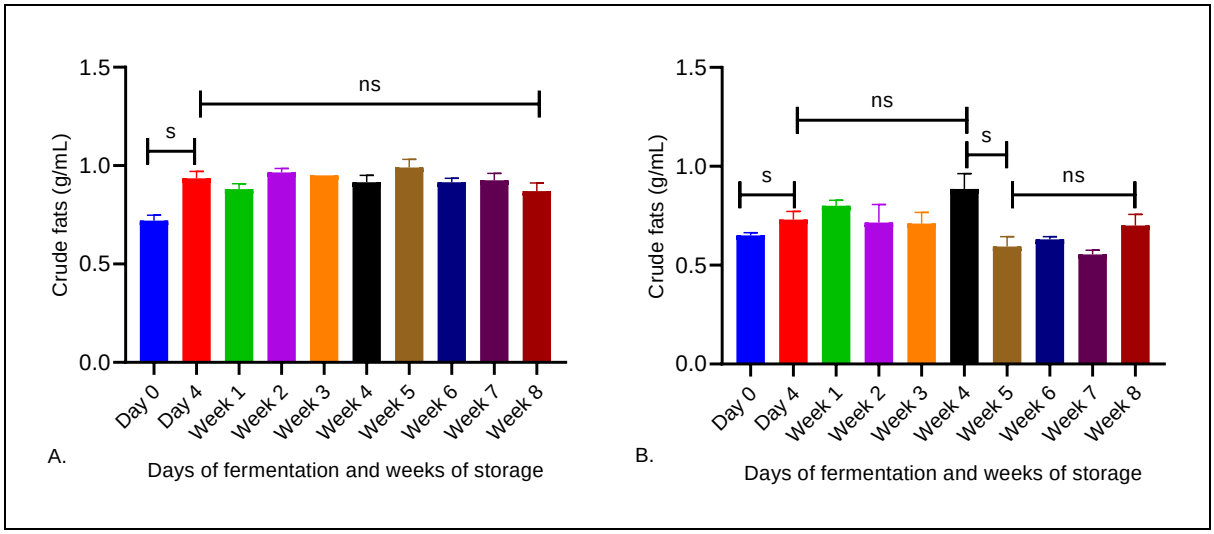

Figure 5.5: Crude fats of 9:1 (A) and 5:5 (B) proportions of mageu. S denotes statistical significance and ns statistical non-significance.

A significant ($p < 0.05$) increase in fat content was noted during fermentation (Day 0 to day 4) in both preparations of millet-based mageu. The crude fat content ranged from 0.84 – 0.96 g/mL in mageu 9:1 and 0.54 – 0.80 g/mL in mageu 5:5 during the storage period. A non-significant increase in the amount of crude fat was noted for the mageu 9:1 (Fig.5.5A) while a significant decline was observed for mageu 5:5 (Fig. 5.5B) after four weeks of storage at 4 °C.

5.3.2.3. Moisture content

The moisture content for the 9:1 proportion of mageu ranged from 85 – 99% with a slight decline in the first week of storage at 4 °C (Fig. 5.6A). The moisture content of the 9:1 preparation of millet mageu was slightly higher than the mageu 5:5, which ranged between 88 – 90% from fermentation and through the storage period.

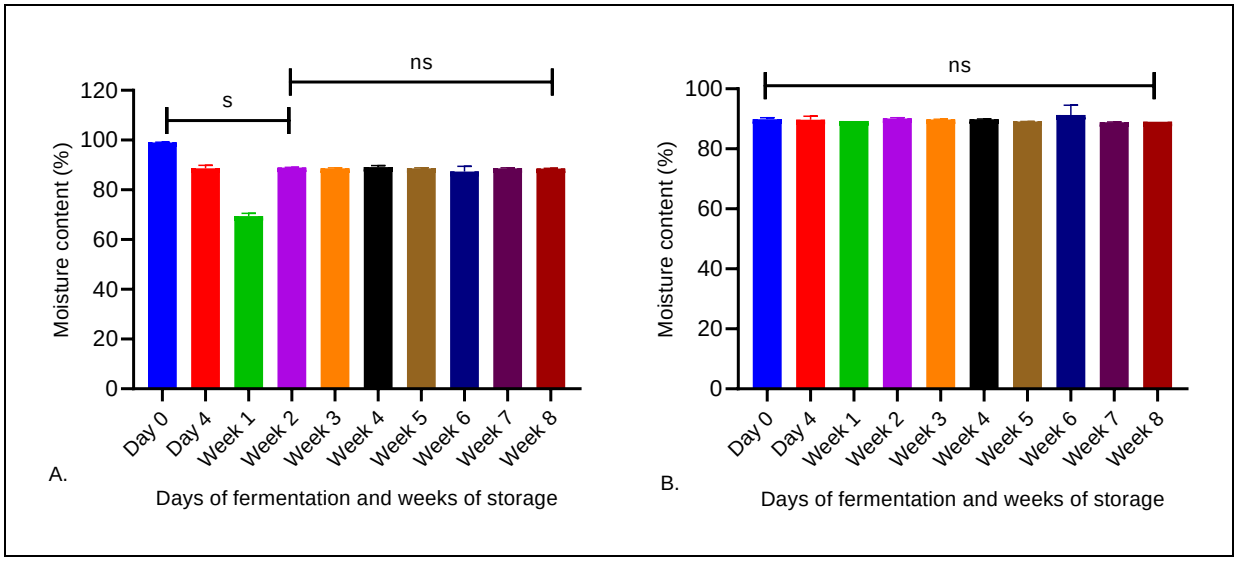

Figure 5.6: Moisture content of both mageu proportions, mageu 9:1 (A) and mageu 5:5 (B). S denotes statistical significance and ns statistical non-significance.

The moisture contents of both mageu preparations remained constant throughout the storage period at 4 °C.

5.3.2.4. Ash content

The ash content (%) of mageu is indicated in Fig. 5.7.

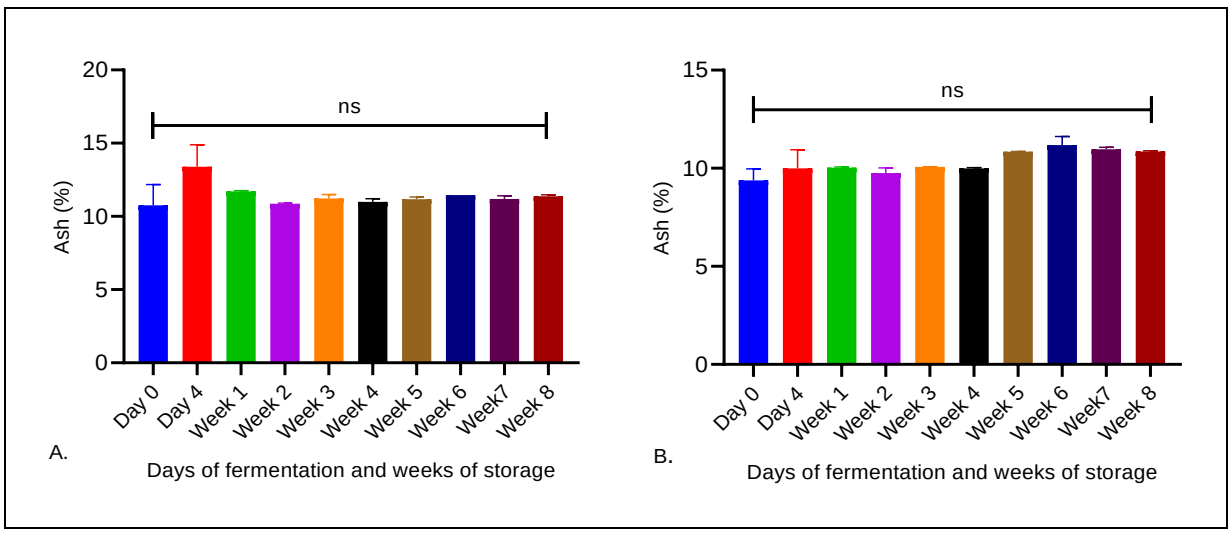

Figure 5.7: Ash % of mageu 9:1 (A) and mageu 5:5 (B). NS statistical non-significance.

Mageu 9:1, which contained more millet component, had a slightly higher total mineral content that ranged from 10 – 13% while the mageu 5:5 ranged from 9.8 – 11% for fermentation and throughout the storage phase.

5.3.2.5. Mineral composition

The presence of minerals such as Ca, K, Mg, Na and P in mageu were noted, whereas Cu, Fe, Mn and Zn were not detected (Fig 5.8). A generally higher amount of all the minerals in the mageu 9:1 proportion was observed when compared to the mageu 5:5 proportion. All the minerals increased significantly ($p < 0.05$) during fermentation (Days 0 to 4) except for P which had a non-significant ($p > 0.05$) increase. The nutritional elements for mageu 9:1 ranged as follows: K from 5.1 – 9.6 mg/mL, P from 6.2 – 8.8 mg/mL, Ca from 1.32 – 4.37 mg/mL, Mg from 1.2 – 4.4 mg/mL and Na from 2.2 – 3.2 mg/mL while for the mageu 5:5, K was the highest at 3.4 – 8.5 mg/mL followed by P at 4.7 – 7.2 mg/mL, Ca at 1.2 – 3.6 mg/mL, Mg at 1.4 – 2.1 mg/mL and Na at 1.4 – 3.1 mg/mL, throughout fermentation and storage, respectively.

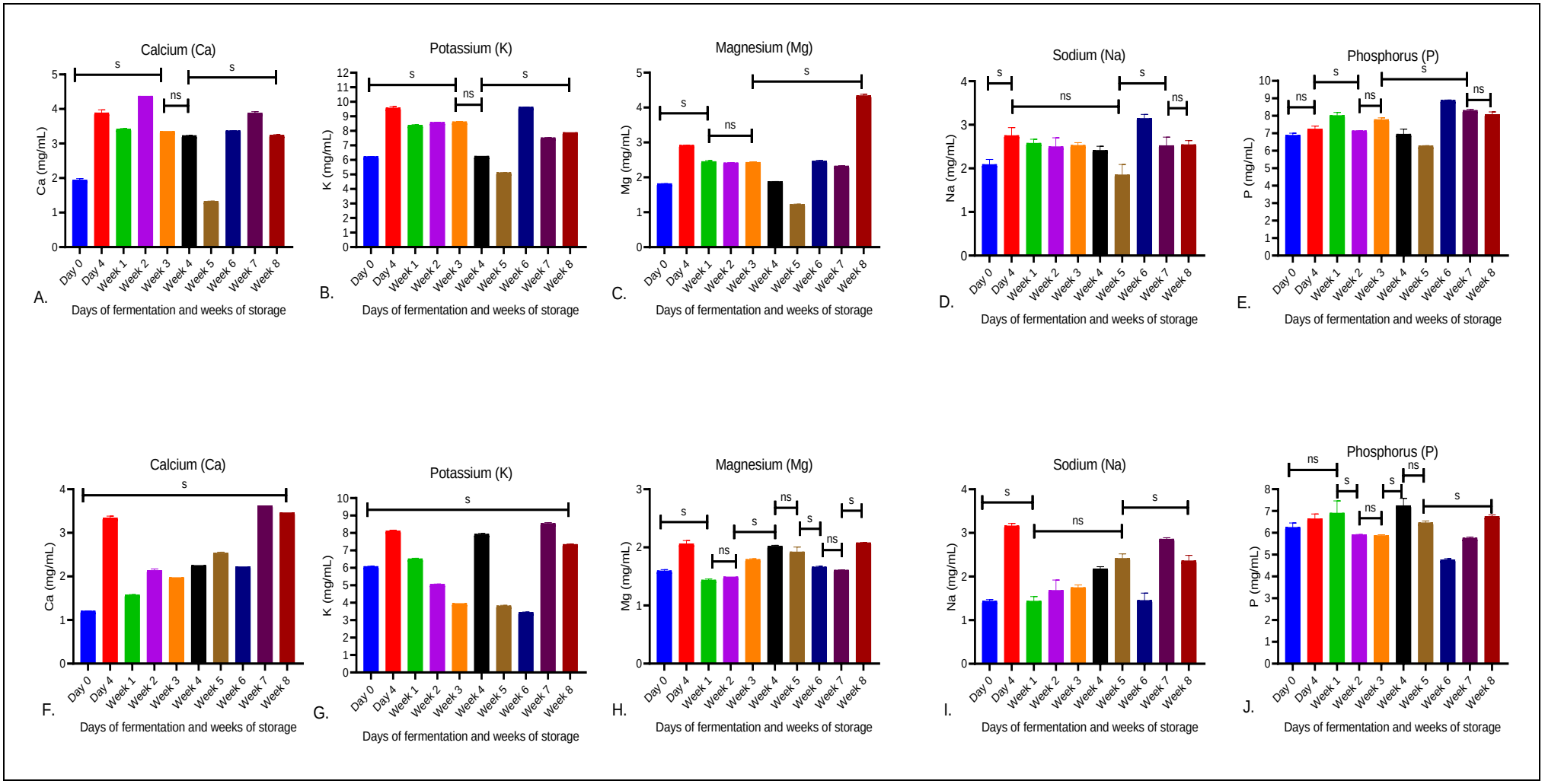

Figure 5.8: Minerals detected in mageu proportions. Minerals in A, B, C, D and E are for millet-based mageu 9:1 and F, G, H, I and J are for mageu 5:5 proportion. S implies statistical significance and ns statistical non-significance.

5.3.2.6. Sugar

Sugars such as sucrose and glucose were detected in both mageu preparations, while fructose was not detected (Fig 5.9).

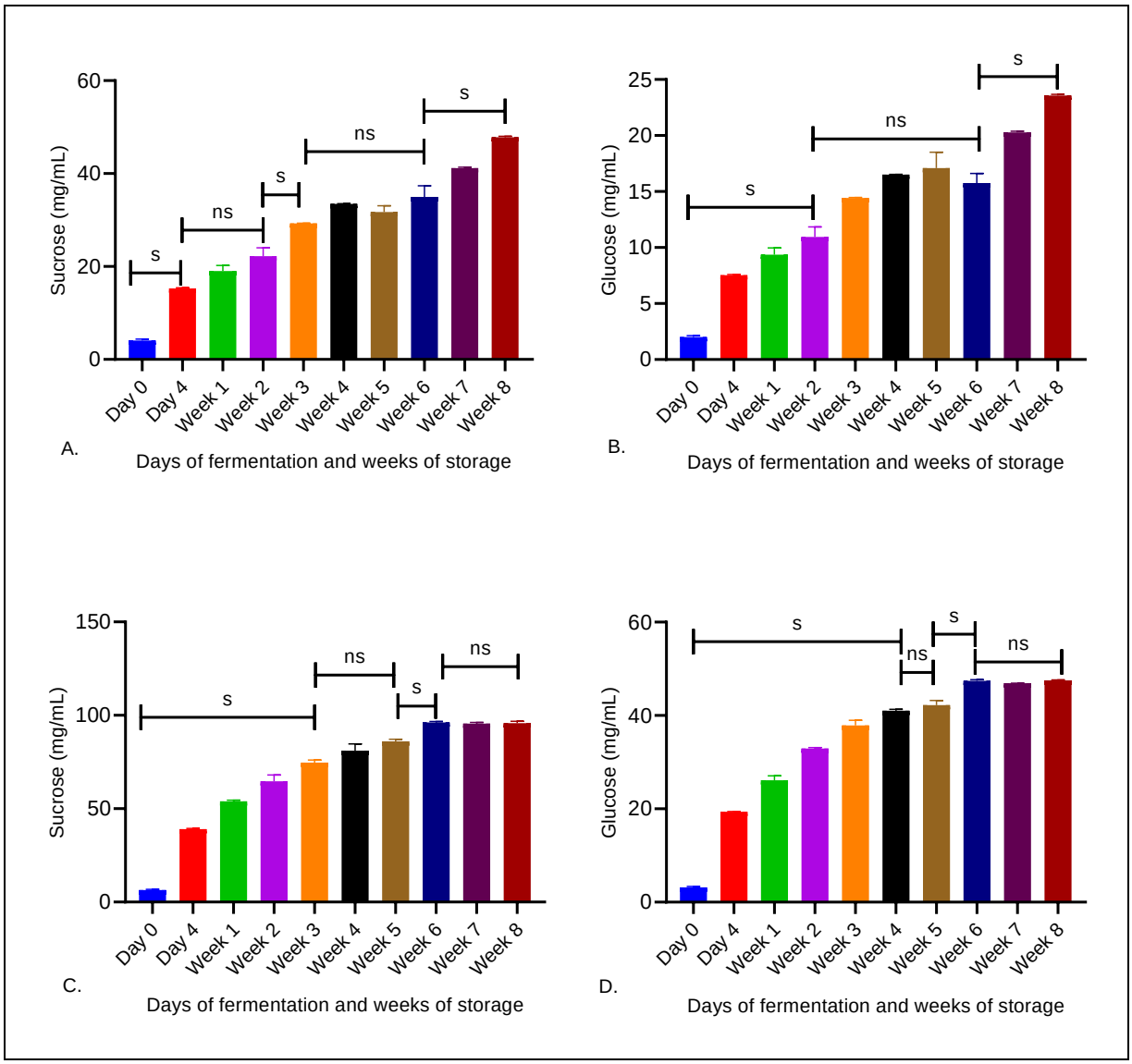

Figure 5.9: Sugar content of millet mageu preparations. Sucrose (A) and glucose (B) in millet mageu 9:1; and sucrose (C) and glucose (D) in mageu 5:5. S denotes statistical significance and ns statistical non-significance.

An initial significant ($p < 0.05$) increase in sucrose and glucose contents was observed during fermentation for both mageu preparations. This was followed by a steady increase ($p > 0.05$) over the course of storage at 4 °C in both mageu proportions (Fig. 5.9). Sucrose ranged from 4 – 47 mg/mL and glucose from 2 – 23.6 mg/mL in the

mageu 9:1 while sugar was at 6 – 96 mg/mL for sucrose and glucose from 3 – 47 mg/mL in the mageu 5:5. This infers a higher sugar content in mageu 5:5. Overall, the gradual accumulation of sugars infers higher degradation rate of carbohydrates accompanied by less consumption of sugars by the fermenting bacteria.

Overall, the millet-based mageu 9:1, which had a higher component of millet, had a higher mineral content and crude fat content. However, there was no notable difference in protein content between the high and low millet-based mageu preparations. The high millet mageu (9:1) showed a proportionally higher organic acid content, an inverse proportion to the pH values observed. This pattern was in line with the amount of sugars detected in the mageu samples, wherein the glucose and sucrose levels were proportionately lower in the high millet content mageu (9:1) than in the low-millet content mageu (5:5). Interestingly, the sugar content was gradually increasing during storage at 4 °C, while fructose was remarkably not detected in both mageu preparations.

5.4. Discussion

The current chapter outlines the chemical and nutritional parameters of millet-based mageu. Mashau *et al*. (2020) previously reported that the difference in the chemical and nutritional composition depend on the type and amounts of cereal grains used and its production conditions, the type of water and utensil used, the types of microorganisms that drive the fermentation process and the length of fermentation incubation period.

The important indicators of an ongoing and successful fermentation process are pH and total titratable acidity (TTA) (Adebo *et al*., 2018). These two concepts are interrelated especially when analysing and dealing with acidity in food products (Adebo *et al*., 2018). pH is an important determinant in food quality to assess if the acidity level favours microbial growth while TTA predicts how organic acids affect the flavour profile of the product (Harris, 2016). An inverse relationship between pH and titratable acidity was observed in both millet mageu preparations in this study, irrespective of the amount of millet used. It is a common occurrence that pH decreases with an increase in TTA during fermentation (Fadahunsi and Soremekun, 2017). The drop in pH and an

increase in TTA during fermentation could be attributed to the breaking down of available sugars by LAB to produce organic/lactic acids thereby making the environment uninhabitable for other microorganisms (Mashau *et al*., 2020). Other microorganisms including yeasts and mould that were detected in mageu could be acting in concert with LAB in the fermentation process. The low pH in food products serves as a natural preservative, and the growth of potential pathogens and spoilage microorganisms that could cause bad fermentation could become inhibited (Mashau *et al*., 2020). The pH reduction and rapid acidification are considered crucial as they extend the shelf life of the product. According to FN Food safety (2008), most food products are below the neutral pH (< pH 7.0) and most cereal-based fermented food products are between the pH 3 and 4.8. The laboratory produced mageu remained within the recommended pH range of mageu which is pH 3 – 4.5. This is significant to the safety of mageu since most food pathogens such as *Listeria monocytogenes* (optimum pH 7.0), *Salmonella* spp. (optimum pH 7 – 7.5) as well as *Escherichia coli* (optimum pH 6 – 7) will not be able to grow due to unfavourable pH conditions (Public Health England, 2017).

Antioxidants are widely used as dietary supplements due to their ability to impede oxidation of other molecules and to fight against diseases and infections (Adebo *et al*., 2018). There is no formal recommendation or an amount of antioxidants in cereal-based fermented food products that should be consumed daily (WHO, 2021) although for vegetables and fruits, a daily intake of 400 g is recommended (Poljsak *et al*., 2021). The antioxidant activity of millet-based mageu was observed throughout fermentation and storage but it was lower than that of L-ascorbic acid, a well-known strong antioxidant. Naturally, different extractant solvents influence the amount of antioxidants detected in food products (Bae *et al*., 2012; Chigayo *et al*., 2016; Ghazzawi *et al*., 2021). The same holds about the fermentation and storage conditions which also affect the amount of antioxidants in food products. Long storage periods often reduce the amount of antioxidants (Poljsak *et al*., 2021) while the longer period of fermentation enhances the amount of antioxidants (Erskine *et al*., 2023). Of consideration is that lactic acid bacteria are reported to have good antioxidant activity and this can further cause the quantity of phenolic compounds and flavonoids to increase (Erskine *et al*., 2023).

The increase in nutritional composition (proteins, crude fats, moisture content, ash and mineral content) during fermentation is common in most maize-based mageu products (Fadahunsi and Soremekun, 2017; Idowu *et al*., 2016). All the nutritional components of millet-based mageu increased during fermentation and Idowu *et al*. (2016) attribute this to the efficient utilisation and conversion of starch from millet by the fermenting microorganisms. Consumption of foods rich in proteins is required to build strong muscles and can also curb many health-related issues such as kidney failure, osteoporosis, and low blood pressure (SACN, 2015). Although adequate protein daily intake is necessary, it is recommended to be within the range of 30 – 45 grams per day (SACN, 2015). The protein content in millet-based mageu (42 – 58 µg/mL) is higher. Millets are rich in unsaturated fats, especially pearl millet (Chandrashekar, 2010). Fats in raw millet (5%) are higher than the fermented millet-based mageu. The daily intake of fats recommended by World Health Organisation range from 20 to 30 g (WHO, 2017). The millet-based mageu had noticeable fats (0.54 – 0.96 g/mL) content although they were significantly below the recommended daily intake (RDI) range, of which the observations are similar to most maize-based mageu (Fadahunsi and Soremekun, 2017). A higher moisture content in both the proportions of millet-based mageu was noted. Moisture content affect the storability of fermented cereals; a higher moisture content reduces the storage period while low moisture content increases the storage period (Idowu *et al*., 2016). The nutritional quality of the laboratory produced mageu highlighted unique findings when it comes to the proteins, since they were significantly higher as compared to previous reports on lower proteins in maize-based mageu (Fadahunsi and Soremekun, 2017).

The ash content reflects the total amount of nutritional minerals present in food (Maakelo *et al*., 2021). A higher ash percentage (9.8 – 13%) was observed in the millet-based mageu as compared to raw millet (1.2%) reported elsewhere (East African Standard, 2011). Fermentation improved the mineral composition of millet-based mageu, and the amounts comply with the permissible limits for mineral intake (East African Standard, 2011). Generally, the recommended daily intake for the major minerals such as sodium, phosphorus, potassium, and magnesium ranges from 4 – 41 mg/mL (Mathipa, 2022). Iron, copper, manganese as well as zinc are required in lower quantities (0.8 mg/mL) because they are classified as trace elements (Mathipa, 2022). Both the ratio of millet-based mageu contained the major mineral elements

such as calcium, potassium, magnesium, sodium and phosphorus with phosphorus and potassium falling within the range of 1.2 – 9.6 mg/mL, while copper, iron, zinc and manganese were not detected. Zinc, iron, copper, and manganese in most maize-based mageu are detected in smaller amounts (Fadahunsi and Soremekun, 2017; Idowu *et al*., 2016). Copper is a heavy metal and is often regarded as a contaminant or toxin, hence its absence does not affect the nutritional composition (East African Standard, 2011). The world Health Organisation highlighted that sodium and calcium intake of < 5 g per day in mageu products is necessary as this could lower the risk of cardiovascular diseases, stroke, and high blood pressure (Foodstuff, 2021). According to the National Centre for Biotechnology Information (NCBI), functional foods such as fermented mageu prevents guts infestation by bacteria as well as diarrhoea in children (Adebo *et al*., 2018) mainly due to the presence of essential minerals (Armistice and Tafadzwa, 2021). These minerals are significant towards maintenance of good health in humans.

Sugars in maize-based mageu, fortified mageu and starter-culture based mageu is often low due to incomplete starch degradation by the fermenting microorganisms (Armistice and Tafadzwa, 2021; Fadahunsi and Soremekun, 2017; Maakelo *et al*., 2021). Fructose was not detected in both the proportions of millet-based mageu, and sucrose and glucose were notably increasing throughout fermentation and storage period. Longer fermentation period following by storage may bring about release of more sucrose and subsequently an increase in glucose (Nkhata *et al*., 2018). Glucose produced during fermentation and storage serves as a preferred energy source for fermenting microorganisms (Nkhata *et al*., 2018). The recommended daily intake (RDI) for sugars differs for women and children as well as for men (Mbhenyane *et al*., 2005). According to the Government Scientific Advisory Committee on Nutrition (SACN), the recommended daily allowance (RDA) of sugar for women should be 25 g, 35 g for men and 18 g for children (SACN, 2015). These sugars play a significant role towards balancing the organoleptic properties of mageu, especially the sourness of mageu along with providing an individual with a quick jolt of energy (Foodstuff, 2021). This holds true because mageu is commonly consumed as a quick energy food product. However, high amounts of sugar exceeding 38 g are detrimental to human health as they may cause diabetes (especially type 2), increased weight gain, high blood pressure, heart diseases as well as tooth decay (SACN, 2015). Consequently,

amounts of sugar intake lower than 18 g can be dangerous to the health of human beings as they may cause brain damage leading to confusion, death as well as anxiety, hence a balance is important to achieve the normal functioning of the body (SACN, 2015).

Generally, the millet-based mageu 9:1 had a slightly better nutritional composition as compared to the millet-based mageu 5:5 although similarities in terms of other nutritional composition such as antioxidants activity and proteins were noted. The millet-based mageu showed to be a nutritious beverage based on the recommended daily allowance (RDA) for different nutrients and in comparison, to the maize-based mageu (Fadahunsi and Soremekun, 2017). A balanced diet rich in acceptable amounts of antioxidants, fats, proteins, moisture content, ash (inorganic minerals), mineral content as well as sugars is recommended to help balance the immune system (Adebo *et al*., 2018) as well as reducing the chances of getting infections (Boyiako *et al*., 2020). Millet-based mageu showed good potential to provide such nutrients and these were constant during the storage period as well.

CHAPTER 6. SENSORY PROFILING OF A MILLET-BASED MAGEU

6.1. Introduction

Mageu is a fermented non-alcoholic beverage that is made from different ingredients such as maize meal, sorghum, left over maize porridge in a natural fermentation process at ambient temperatures (Mashau *et al*., 2020). It is characterised by the presence of organic acids, low pH, various microorganisms, essential nutrients which all contribute towards the palatability and flavour (taste and smell) (Eswatini, 2019), as well as the colour, texture, and consistency (thickness and thinness) of mageu (Fushiki and Nakano, 2019). Africans across the continent consider mageu as an energy and thirst-quenching beverage (Maakelo *et al*., 2021). Mageu is consumed by all age groups and is often used as weaning food for infants (Idowu *et al*., 2016).

The storage and fermentation conditions (the incubation period and temperature) contribute to the sensorial characteristics of a fermented product (Fushiki and Nakano, 2019). The presence of acids contributes towards the sour taste as well as the smell of mageu. The lactic acid bacteria (LAB) such as *Lactobacillus bulgaricus*, *Lactobacillus brevis*, *Lactobacillus delbrueckii*, *Lactococcus lactis* to name a few, are responsible for the production of lactic acid, which is attributed to the sour odour and taste of mageu (Salvado *et al*., 2016; Wikandari *et al*., 2021). In addition, the changes and differences in the organic acids, pH as well as fat influence the flavour of mageu (Mashau *et al*., 2020). Bioactive compounds such as antioxidants and tannins also add to the sour taste, which is desirable to some of the consumers. The study undertaken by Idowu *et al*. (2016) reported that over 80% of the participants in the study who were aged between 19 – 25 years preferred to drink mageu not only because of its nutritional value but for its unique sour taste.

The chemical reactions between sugars, moisture contents, fats and proteins have an impact towards the colour, texture, and consistency of mageu (Olusanya *et al*., 2020). Various researchers reported on improved consumer acceptability of mageu based on its organoleptic properties (Mashau *et al*., 2020; Obinna-Echem, 2014; Olusanya *et*

al., 2020). The sensorial properties such as flavour, colour, texture, and consistency are important for consumer acceptability, with colour being an important aesthetic value for the consumer (Shindano, 2007). Notably, the colour of most homemade mageu is unknown as it tends to differ due to the materials used (Shindano, 2007).

The types of grains used continually to produce mageu include sorghum and maize meal (Mashau *et al*., 2020), while millet grain is seldom used (Vinoth and Ravindahran, 2017). Millet has a bitter taste that limits its use for production of food products (Vinoth and Ravindahran, 2017). Thus, fermentation of millet might lighten the bitter taste and make the product palatable. Hence, the current study describes the sensorial attributes of mageu produced from pearl millet grains such as taste, colour, smell, texture, consistency, and its overall impression.

Sensory profiling of various food and beverage products are often carried out following blind tasting procedures by either a semi-trained, trained, or an untrained panel for a better understanding of the product performance (Singh-Ackbarali and Maharaj, 2014). The results obtained after application of these methods help the product developers to develop and launch products with higher consumers acceptability (Singh-Ackbarali and Maharaj, 2014).

6.2. Materials and methods

6.2.1. Millet-based mageu preparation and sample collection

Millet-based mageu was prepared following the method of Fadahunsi and Soremekun (2017) with minor modifications. Two proportions, namely, 9:1 and 5:5 were prepared using pearl millet flour in a total volume of 7 L. The proportions were prepared as outlined in chapter 4, section 4.2.1.

6.2.2. Sensory analysis of millet mageu

This protocol received ethical clearance (Appendix A). Sensory profiling was evaluated based on taste, smell, colour, texture, consistency, and overall impression. Tasting was conducted at the University of Limpopo by a semi-trained panel that consisted of six volunteers of different ages (between 20 and 60) and gender (2 males and 4

females). The participants involved the students in certain instances, but the composition remained constant. The panel members completed consent forms prior to tasting. A modified version of sensory evaluation form from Singh-Ackbarali and Maharaj (2014) (Appendix B) was used to capture the impressions by the panellists. Ten millilitres of mageu samples in closed clean transparent cups were used for tasting evaluation at each sampling interval. The participants received potable bottled water and slices of cucumber and carrots as palate cleansers to use in between tasting of different mageu products. The impressions were rated based on a 5 – point hedonic scale (1 = extremely dislike, 2 = dislike, 3 = neutral, 4 = like and 5 = extremely like). Blind tasting was carried out; there were no identifiers about the material and conditions used in the production of the two proportions of mageu.

6.2.3. Statistical analysis

One way ANOVA followed by Tukey's multiple comparisons of graph pad prism 8 build-in analysis program were used for determining the mean average of all the sensorial parameters (colour, smell, taste, texture, consistency, and overall impression) and p value. Significance was determined at $p \leq 0.05$.

6.3. Results

Two millet-based mageu products were prepared by natural fermentation of the millet-maize gruel for four days at 25 °C and thereafter the mageu was stored at 4 °C for 8 weeks. The 9:1 and 5:5 proportions were evaluated for their sensorial properties based on colour, aroma, taste, texture, consistency as well as overall impression.

6.3.1. Colour

Both mageu preparations were described by the tasting panel as a cream to brownish colour at the end of fermentation (Day 4); and similar hue in the range of brown, light brown, yellow-brown and cream white was reported for mageu throughout the storage period as indicated in Fig 6.1.

The colour for the mageu 9:1 was scored from 3.3 – 4.5 (Fig 6.1A). There was no significant difference ($p > 0.05$) in the scores recorded by the panellists for both millet-

based mageu proportions (Fig 6.1). The creamy whitish colour was dominant for mageu 5:5 The disparity was in the colour of mageu 5:5 which was described as grey and milkish grey during the storage period. The colour descriptors were dissimilar on day 4 and period of storage for the 5:5 mageu. The 5:5 mageu values ranged from 3.6 – 4 (Fig 6.1B).

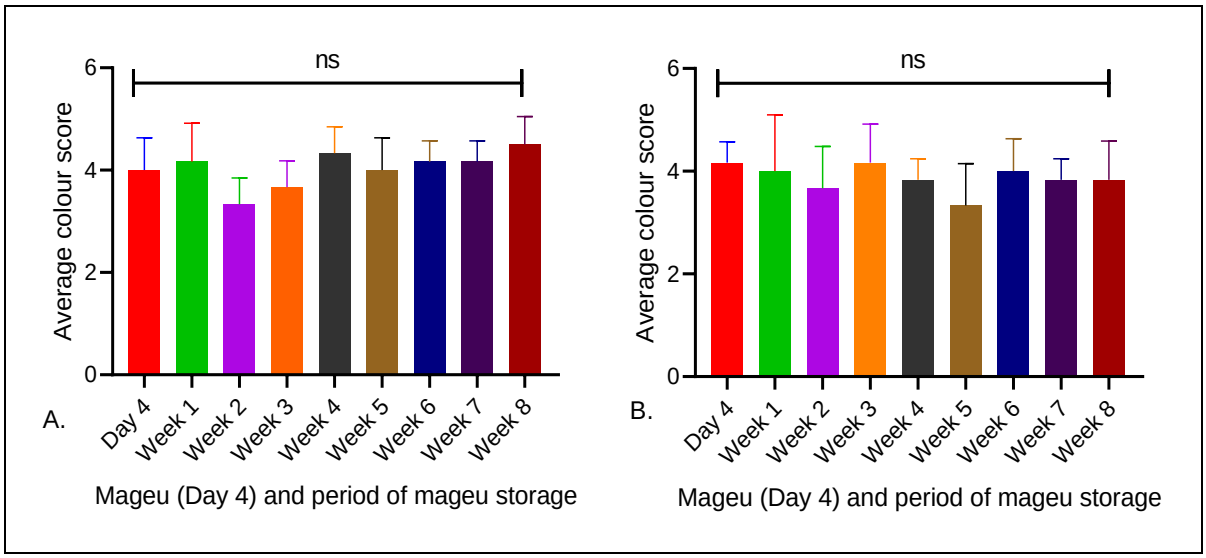

Figure 6.1. Average colour score of millet-based mageu 9:1 (A) and millet-based mageu 5:5 (B). NS denotes statistical non-significance.

The panellists liked the colour of mageu 9:1 in comparison to mageu 5:5.

6.3.2. Aroma

The panellists liked the aroma of millet-based mageu 9:1 more at an average score that ranged from 2.8 – 3.8 as compared to the average range of mageu 5:5 at 3.16 to 3.6 (Fig 6.2).

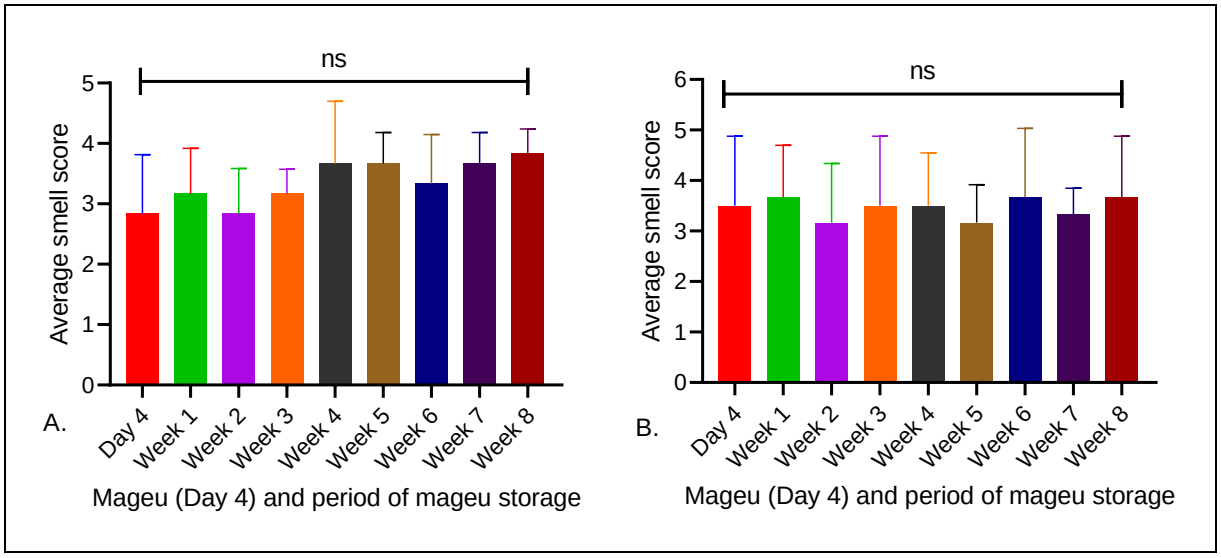

Figure 6.2. Average smell score of millet-based mageu 9:1 (A) and millet-based mageu 5:5 (B). NS denotes statistical non-significance.

The smell of millet-based mageu 9:1 was described as commercial mageu, incomplete fermentation, fermented porridge, and boiled corn on day 4, which marked the end of fermentation process while throughout storage the smell was pronounced as an acidic aroma. On day 4 of mageu 5:5 production, the aroma was described as fruity, bronzy, and acidic. Aroma was described as tart, strong sweeter aroma with a touch of commercial mageu smell during weeks 1 – 4 of storage, while in weeks 5 – 8, the mageu aroma was consistently described as an acid smell for the mageu 5:5.

6.3.3. Taste

The taste score for millet-based mageu 9:1 ranged from 2.5 – 3.6 and 3.3 – 4.3 for the millet-based mageu 5:5 (Fig 6.3). The statistical non-significant ($p > 0.05$) difference was noted in both the proportions of mageu (mageu 9:1 and mageu 5:5) throughout even though the panel gave non-comparable taste descriptions. Notably, the millet-based mageu 5:5 was liked the most compared to the millet-based mageu 9:1 (Fig 6.3).

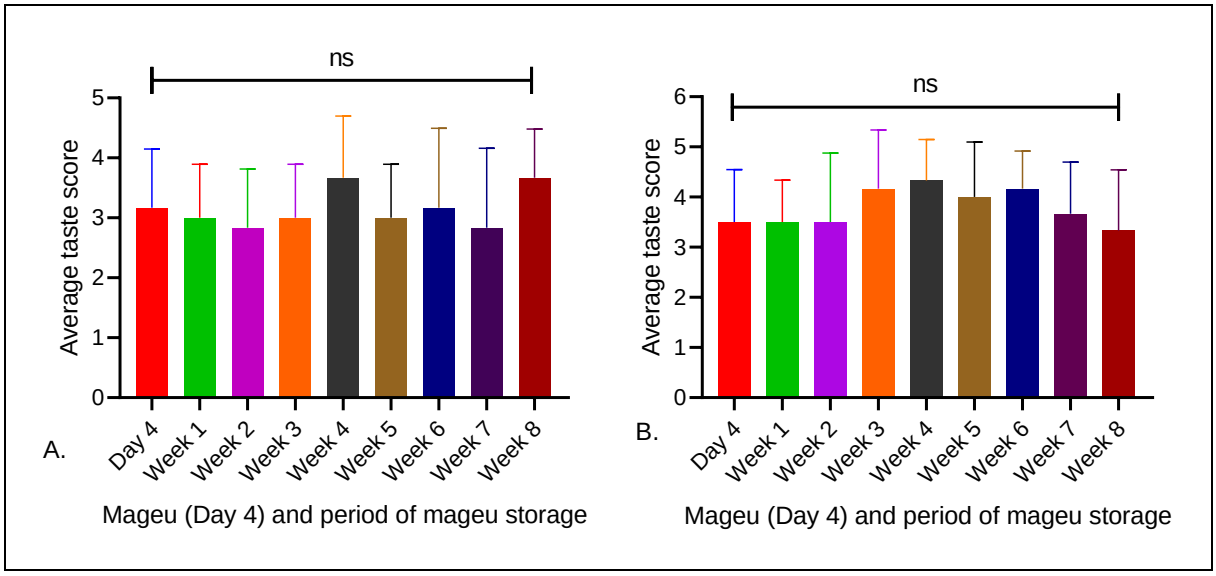

Figure 6.3. Average taste scores of millet-based mageu 9:1 (A) and millet-based mageu 5:5 (B). NS denotes statistical non-significance.

The panel described the taste of millet-based mageu 9:1 as sour, sour-sweet, creamy sour, fermented porridge like, and corn-like notes at the end of fermentation, whereas during storage mageu 5:5 was recorded as bitter, sour-bitter with a sour taste being chosen mostly. The taste character for millet-based mageu 5:5 on day 4 of fermentation was noted as mild sour, mild creamy, sweet, sweet bitterness and sour fruity while the taste was described as sour-sweet, slightly acidic, sweet-woody, fruity with an acidic finish during storage.

6.3.4. Texture

Millet-based mageu 5:5 texture was constant throughout and it was perceived as smooth. The mageu 9:1 texture was described as chewy and silky, with rough texture being the dominant descriptor throughout. Although the texture descriptors were distinct on day 4 and during storage, the score values by the panellists were non-significant ($p > 0.05$) (Fig 6.4).

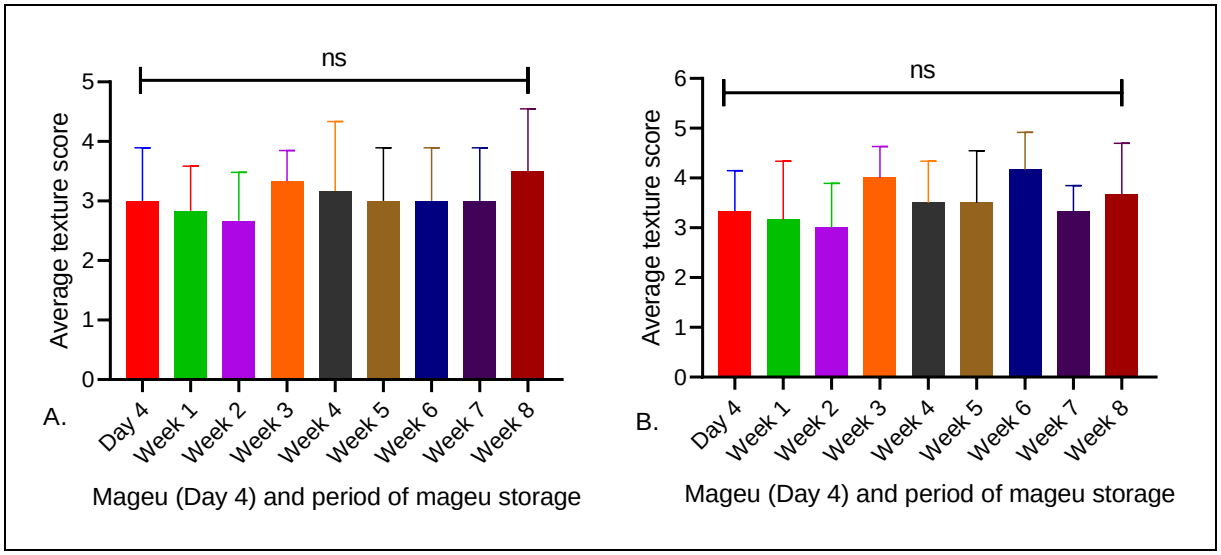

Figure 6.4. Average texture scores for mageu 9:1 (A) and mageu 5:5 (B). NS denotes statistical non-significance.

The panellists liked the millet-based mageu 5:5 more than the millet-based mageu 9:1 with a score range of 3.16 – 3.5 compared to 2.6 – 3.5 for millet-based mageu 9:1.

6.3.5. Consistency

The panellists described the consistency of millet-based mageu from fermentation to week 3 of storage as watery and thin for both proportions of mageu (mageu 9:1 and mageu 5:5). For subsequent weeks 4 – 8 of storage, the millet- based mageu 9:1 was noted as semi-fluid and likened to the commercial mageu, although most panellists still recorded a watery consistency. The consistency of millet-based mageu 5:5 was perceived as very liquid and watery from weeks 4 – 8. Whilst the consistency descriptors were not the same throughout for mageu 9:1 and mageu 5:5 (Fig 6.5), the panel scores were non-significant ($p > 0.05$) throughout.

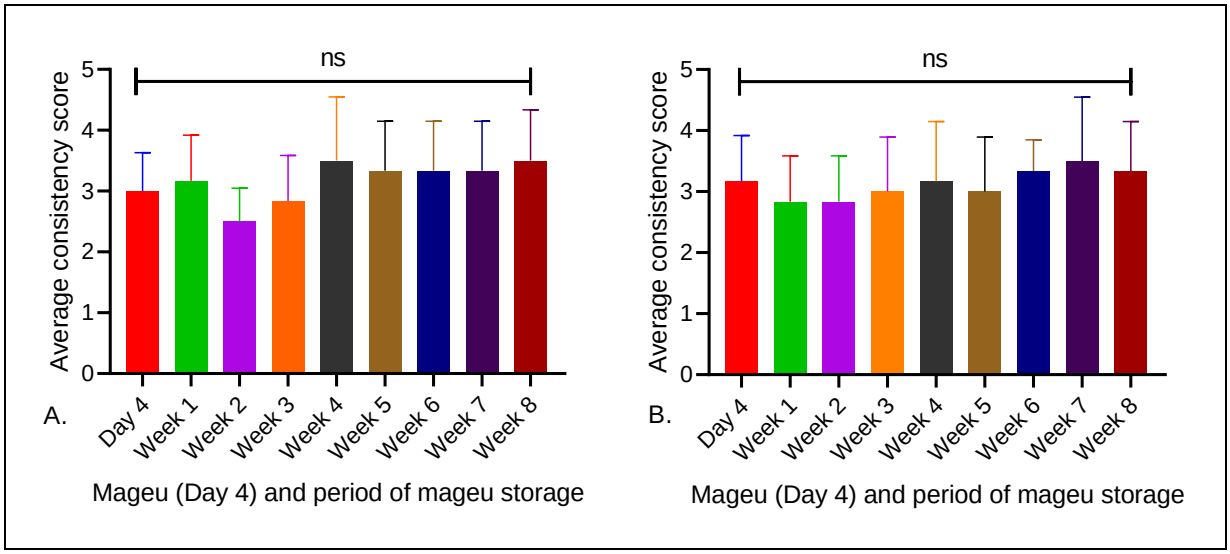

Figure 6.5. Average consistency score of mageu 9:1 (A) and mageu 5:5 (B). NS denotes statistical non-significance.

The scores fluctuated within a range of 2.5 – 3.6 for mageu 9:1 and 2.8 – 3.5 for mageu 5:5. The panellists preferred the consistency of millet-based mageu 9:1 over millet-based mageu 5:5.

6.3.6. Overall impression

The overall impression parameter enquired on whether the tasting panellists appreciated the product and would buy it. The mageu 5:5 had the better overall appreciation at a score range of 3.16 – 4 than the mageu 9:1 with a range of 2.4 – 3.5 (Fig 6.6).

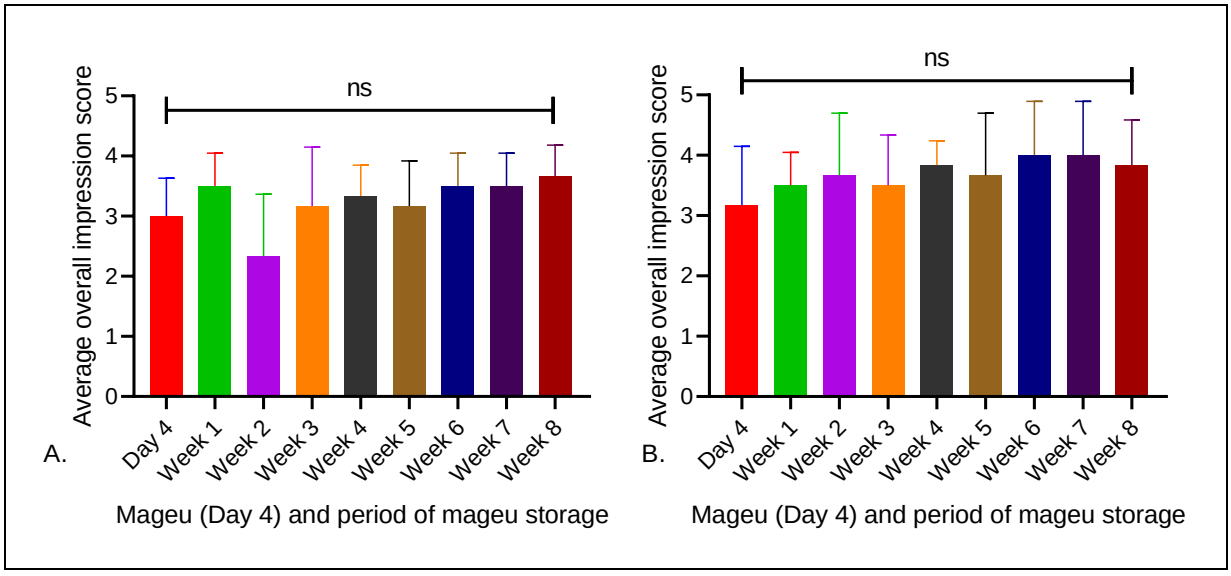

Figure 6.6. Overall impression average scores for millet-based mageu 9:1 (A) and millet-based mageu 5:5 (B). NS denotes statistical non-significance.

Although the panellists generally appreciated the mageu 5:5, the millet-based mageu 9:1 was also appreciated from weeks 4 – 8. The overall impression of millet-based mageu was influenced by taste, colour, aroma, texture, and consistency. The sensory attributes above indicated the non-significant ($p > 0.05$) difference throughout for all the proportions corresponding to the overall impression.

6.4. Discussion

This chapter focused on outlining the sensory attributes of millet-based mageu. Colour is important as the first characteristic that appeals to the consumers; it influences product acceptability. The colour of the two millet-based mageu proportions differed throughout the fermentation and storage period. Pearl millet contains various phytochemicals such as tannins and polyphenols. These phytochemicals influence the colour of millet during cooking, fermentation and storage (Dayakar *et al*., 2017). The millet retains the original colour at higher temperatures around 25 – 30 °C due to the presence of a brown pigment and becomes light brown when the temperatures are lowered especially during storage between 4 °C – 15 °C (Dayakar *et al*., 2017). The levels of tannins and polyphenols decrease during fermentation, thus affecting the brown pigment of millet (Chandrashekar, 2010). This explains the colour differences in the two mageu products with different millet proportions. Nonetheless, both millet-

mageu products attained a lighter shade (light cream, light brown, yellow brown, cream white, grey, milky) during fermentation and storage. Consumers prefer light-coloured foods as the colour influences their opinion about the quality of the food products (Sahin and Sumnu, 2006).

The absence of alcohol in mageu imparts sweet and fruity aromas (Moodley, 2019). Fermenting bacteria produce organic acids as the main fermentation product. As such, the dominant fermenting bacteria like the LAB produce mainly lactic acid (Wikandari *et al*., 2021) which could be attributed to the acidic taste and aroma in mageu 9:1 and the tart smell in mageu 5:5 during storage. It is noteworthy to mention that saponins are not removed during fermentation and the saponin coating found in millet grains is responsible for the bitter taste of millet (Kumar *et al*., 2021; Sarita and Singh, 2016). Natural attributes of the ingredients also contribute to the sensory profile of the product. Different proportions of maize meal and millet powder were used in the millet mageu preparations. Unsurprisingly, the aroma notes such as maize meal smell, fermented porridge and boiled corn were recorded in this study and these could be attributed to the amount of millet flour used for preparations of mageu and the activity of the fermenting microorganisms (Fadahunsi and Soremekun, 2017). Furthermore, degradation of amino acids and lipids during microbial fermentation are known to influence the aroma of fermented foods as well (Moodley, 2019). The aroma of food products plays an important role as it orients consumer appetite (Boesveldt and Parma, 2021) and the balance between colour and aroma has a huge impact towards food intake (Boesveldt and Parma, 2021).

Generally, fermentation adds value to food (Mashau *et al*., 2020). Fermenting microorganisms play a major role towards texture of cereals by improving it, thereby making these cereals smooth and creamy in texture (Mashau *et al*., 2020). Moreover, it was reported that the nutritional composition often influences the texture, especially the high fat and moisture contents (Zanra *et al*., 2022), which often softens the product (Shin *et al*., 2019). Pearl millet flour has a higher lipid content which is high in unsaturated fatty acids (Dayakar *et al*., 2017). The rough texture of mageu commonly results from the non-hydrolysed starch granules during fermentation (Mashau *et al*., 2020) while the soft texture could be attributed to the high fat content and hydrolysed starch granules. LAB produces proteolytic enzymes mostly at pH 7 to 3.5, especially

proteases which are responsible for the thickening of several cereal-based fermented products and other soft drinks (Shin *et al*., 2019). Furthermore, several studies have demonstrated that the levels of proteins tend to be lower in fermented cereals, and this consequently affect the consistency of mageu (Olusanya *et al*., 2020).

The balance between colour, smell, taste, texture, and consistency has a huge impact towards product acceptability, its quality as well as reproducibility (Boesveldt and Parma, 2021). The overall impression revealed that the tasting panel appreciated the millet-based mageu 5:5 which contained high millet used as the inoculum but less in total content compared to mageu 9:1 that had an overall higher millet proportion. Interestingly, members of the tasting panel (in all the age groups) who consume alcoholic beverages and regular consumers of commercial mageu preferred the mageu 9:1 due to its sour/bitter taste while non-regular consumers of mageu preferred the mageu 5:5 because of its sweet-sour taste. Based on the study findings, the combination of millet and maize meal using different proportions produces mageu (millet-based mageu) with unique sensorial attributes that can accommodate different kinds of consumers.

CHAPTER 7. GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

7.1 General discussion

Millet grains are in abundance with exceptional nutritional composition, which promotes good health (Pradeep and Sreerama, 2018). Millet grains are adaptable to unfavourable weather conditions in contrast to maize that is currently depleting (Adebiyi *et al*., 2018). The study explored the introduction of millet grains as an alternative cereal meal to maize and sorghum in the making of the traditional fermented non-alcoholic beverage called mageu. Mageu is known for its good health benefits and nutritional significance which are beneficial in mitigating the effect of malnutrition and food insecurity (Eswatini, 2019). Mageu is consumed as breakfast or energy drink by both children and adults in most rural African communities (Eswatini, 2019). It is produced through natural fermentation using maize meal or left-over maize porridge and sorghum at ambient temperature (25 °C). The current study revealed that the production of mageu using millet meal at different proportions consisted of lactic acid bacteria (LAB) that possess probiotic potential and health benefits. Moreover, millet-based mageu showed increased nutritional compositions with a prolonged shelf life of up to two months and good sensorial attributes.

Literature has reported that maize based mageu has LAB belonging mostly to the *Lactobacillus* genus (Idowu *et al*., 2016; Salvado *et al*., 2016). This contrasted with the findings in this study that showed the dominance of LAB belonging to the *Pediococcus* genus; namely *Pediococcus acidilacti* (coded MD02) and *Pediococcus pentosaceus* (coded MD01). It could be that the LAB belonging to the *Pediococcus* genus originated from the millet grains that were used as the initiator of fermentation. In most maize-based fermented beverages, L*actobacillus* genus dominates. LAB belonging to either the *Lactobacillus* genus or *Pediococcus* genus have probiotic potential. Moreover, the *Pedioccoccus* genus tends to aggressively produce higher amounts of lactic acids while the *Lactobacillus* genus gently produce lower amounts of lactic acids (Garcia *et al*., 2016). LAB in the millet-based mageu proportions were higher than the total aerobic bacteria, yeasts and moulds and such findings indicate that LAB were the main

drivers of fermentation. This is congruent with other studies (Garcia *et al*., 2016). Importantly, the World Health Organisation reported that LAB are generally recognised as safe (GRAS) (Anitha *et al*., 2018). Probiotics, which are mostly LAB strains, are regarded as the good bacteria that offer good health benefits to humans by maintaining a microbial balance in the stomach and the intestinal track thereby preventing possibilities of intestinal infections and diseases such as diarrhoea (Bonatsou *et al*., 2017). Furthermore, the resistance of bile secretions by the LAB is one of their most important characteristics which enable them to survive and thrive in the intestines (Oh and Jung, 2015). *P. acidilacti* MD02 and *P. pentosaceus* MD01 exhibited good probiotic attributes since they were able to withstand a low pHs of 2.5 and 3 as highlighted by the survival rate ranging from 80% to 90%. These strains further showed the ability to survive and tolerate the bile conditions of the stomach (40% – 90% survival rate), both at low concentrated (0.3%) and high concentrated (0.9%) bile conditions. The ability of *P. acidilacti* MD02 and *P. pentosaceus* MD01 to survive under harsh conditions of the stomach is important for creating a healthy balance in the intestines and the stomach (Reller *et al*., 2009). Both the LAB strains showed resistance to at least two antibiotics, streptomycin and penicillin that are commonly used as first line of treatment against bacterial infections in health facilities (Jahantigh *et al*., 2020). Their resistance is an indicative that these strains will continue to grow in the gut of the individuals who consume mageu even when the individual is taking antibiotic treatment. Another health property displayed by the *P. acidilacti* MD02 and *P. pentosaceus* MD01 isolates was the moderate antioxidant activity, which could benefit the health of humans by improving their innate immunity as well as improving mucosal proliferation (Wang *et al*., 2021).

The total titratable acidity (TTA) of millet-based mageu and pH showed an inverse relationship which played a huge role towards balancing the microbial populations of mageu as well as providing the safety of the product, thereby prohibiting the growth of unwanted microorganisms such as *E. coli* (Public Health England, 2017). A low pH in fermented food products serves as a preservative measure (Public Health England, 2017) thereby increasing the shelf life of the products (Idowu *et al*., 2016). Inadvertently, a prolonged period of storage might have affected the antioxidant activity of millet mageu since the amount of antioxidant activity was satisfactorily high or increasing before and during fermentation. The pearl millet meal improved the

nutritional composition of millet-based mageu with regard to minerals and sugar content (glucose and sucrose). High nutritional contents are beneficial towards improving the health of human beings (Foodstuff, 2021). Generally, a balanced diet containing all the desirable nutritional contents gives an individual all the required energy (Foodstuff, 2021).

The differing proportions of millet grains used as an inoculum played a significant role towards the sensorial properties (colour, smell, taste, texture, and consistency) of millet-based mageu. It is noteworthy that the balanced flavour (smell and taste), colour, texture and consistency of a food product orients appetite as well as influencing food intake (Boesveldt and Parma, 2021). Millet grains have saponin coating that is responsible for their bitter taste (Kumar *et al*., 2021; Sarita and Singh, 2016). This bitter taste might have been neutralised by fermentation considering that the mageu products tasted sour-sweet and sweet taste and organic acids produced by LAB and other bacteria during fermentation contributed to this taste. The use of millet (pearl millet) in fermented mageu beverage and possibly other fermented food products has great potential based on the nutrient richness and good probiotics the mageu product displayed in this study. Furthermore, fermentation is a good food production strategy as it produced safe food products. *Escherichia coli* is recognised as a food pathogen (Ekici and Dumen, 2019) and its absence during the production of millet-based mageu is a desirable outcome of fermentation process.

7.2 Conclusion

The investigation of the potential of millet as an alternative cereal to maize yielded positive results. This was premised on the common positive effects of fermentation, that is, in improving the (i) nutritional composition, (ii) the sensory profile (iii) health potential of the mageu products produced from the two proportions of millet-based mageu. The longer shelf life was inadvertently one of the highlights of the study. Both millet-based mageu remained stable in chemical and sensory profiles for eight weeks (two months). These findings affirm the importance of millet as an alternative cereal to maize and sorghum that is available and more nutritious.

7.3 Recommendations

Future studies involving the use of combined starter cultures from *Lactobacillus* and *Pediococcus* to produce mageu will be beneficial in terms of microbial dosage and augmentation of the beneficial properties observed. However, it will be important to study the fermenting lactic acid bacteria *Pediococcus* strains to fully explore their capabilities noting the possibility of these being sub-strains with novel characteristics.

CHAPTER 8. REFERENCES

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APPENDIX A

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TURFLOOP RESEARCH ETHICS COMMITTEE

ETHICS CLEARANCE CERTIFICATE

MEETING: 08 December 2021

PROJECT NUMBER: TREC/325/2021: PG

PROJECT:

Title:	Evaluation of the Microbiological, Nutritive and Health Properties of a Millet-Based Fermented Beverage
Researcher:	D Mogashoa
Supervisor:	Prof KLM Moganedi
Co-Supervisor/s:	Prof EL Jansen Van Rensburg
School:	Molecular and Life Science
Degree:	Masters of Science in Microbiology.

PROF P MASOKO
CHAIRPERSON: TURFLOOP RESEARCH ETHICS COMMITTEE

The Turfloop Research Ethics Committee (TREC) is registered with the National Health Research Ethics Council, Registration Number: **REC-0310111031**

Note:

i) **This Ethics Clearance Certificate will be valid for one (1) year, as from the abovementioned date. Application for annual renewal (or annual review) need to be received by TREC one month before lapse of this period.**

ii) **Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee, together with the Application for Amendment form.**

iii) **PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.**

Finding solutions for Africa

APPENDIX B

Sensory Evaluation Form of millet-based mageu non-alcoholic beverage

Date:_____

Identification:_____ **Colour code of the sample:** _____

Gender:

Female		**Male**		**Other**	

Age group:

Under 21		**21-30**		**31-40**	
41-50		**51-60**		**Over 60**	

You have been given samples to evaluate. Please taste the samples in order presented. Indicate how much you like or dislike the sample by rating it on a scale of 1-dislike extremely to 5-like extremely. Please eat a carrot and cucumber as well as drinking water in between the tasting.

Sample number	Sensory attributes					
		1. Extremely dislike	2. Dislike	3. Neutral	4. Like	5. Extremely like
	Colour/ Mmala					
	Smell/ Monkgo					
	Taste/ Tatso					
	Texture/ Mohlodi					
	Consistency/Kelelo					
	Overall impression/ Kgahlego					
Would you buy it? /O ka e reka?					Yes	No

Comments:

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