UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Nutritional, antioxidant and microbiological properties of finger millet-based beverages as influenced by starch source during lactic acid fermentation

By

Lungani A. Nyathi

Submitted in partial fulfilment of the requirements for the degree

MSc (Food Science)

In the Department of Consumer and Food Sciences

Faculty of Natural and Agricultural Sciences

University of Pretoria

South Africa

June 2021

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

DECLARATION

I hereby declare that this dissertation submitted at the University of Pretoria for the award of the degree MSc. Food Science is my own work and has not previously been submitted by me for a degree at this or any other university or institution of higher education.

Lungani A. Nyathi

June 2021

DEDICATION

This dissertation is dedicated to my parents. Thank you for never giving up on me and for always being my source of inspiration.

To God Almighty who makes everything beautiful in its time (Ecclesiastes 3:11).

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to:

My research supervisor, Prof. K. G. Duodu, for the opportunity to carry out this research, providing exceptional guidance, encouragement and his patience throughout the research.

My co-supervisors Prof. E. M. Buys and Prof. H. C. Schönfeldt for their well-needed guidance, and constructive criticism that improved the research.

Dr. Srinivasan Krishnamoorthy, Mr. Njowe Kieran Yisa, Mr. Selorm Agbemavor and Mr. John Lubaale, I am truly grateful for your assistance and suggestions during the research.

Family, friends, staff, and fellow postgraduate students of the Department of Consumer and Food Sciences, University of Pretoria for their support, positive criticism and guidance during this study.

The author acknowledges funding from the Department of Science and Technology (DST)/National Research Foundation (NRF) South African Research Chairs Initiative (SARChI) in the National Development Plan Priority Area of Nutrition and Food Security (Unique number: SARCI170808259212). The grant holder acknowledges that opinions, findings and conclusions or recommendations expressed in any publication generated by the NRF-supported research are that of the author, and that the NRF accepts no liability whatsoever in this regard.

Finally, my rock and strength God Almighty! Thank you for the intellectual capacity, none of this would have been possible without you in the field!

ABSTRACT

Nutritional, antioxidant and microbiological properties of finger millet-based beverages as influenced by starch source during lactic acid fermentation

By

Lungani A. Nyathi

Degree: MSc. Food Science

Supervisor: Prof. K. G. Duodu

Co-supervisors: Prof. E. M. Buys

Prof. H. C. Schönfeldt

In Sub-Saharan Africa (SSA), where nutrient malnutrition is of concern, finger millet contributes substantially to the dietary protein and mineral intake of many communities. The quality of finger millet protein is, however, poor due to deficiency of essential amino acids like lysine and reduced digestibility due to complexation with antinutrients such as phytate which can also reduce mineral bioaccessibility. Despite the nutritional challenges, finger millet is rich in bioactive phenolic compounds which are gaining increased research focus due to the potential health benefits they can offer in terms of protection against diet-related non-communicable diseases (NCDs). Various fermented beverage products are consumed in SSA, where the fermentation process involves inclusion of an exogenous source of starch. Crucially, the fermentation process can modify the microbial population, nutrients, and bioactive compounds. This presents an opportunity for the improvement of cereal-based diets to leverage their nutritional and health benefits fully. In this study, the influence of starch source- maize and rice- on protein quality, mineral bioaccessibility, phenolic content, and antioxidant and microbiological properties of fermented finger millet-based beverages was determined.

Finger millet slurries were prepared by spontaneous fermentation with or without addition of exogenous starch from maize or rice, whilst beverages were prepared by cooking finger millet slurries. Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) and 16S ribosomal ribonucleic acid (16S rRNA) amplicon sequencing were used to assess microbial diversity of slurries, whilst *in vitro* dialysability, *in vitro* protein digestibility (IVPD), and reactive (available) lysine assays were done on slurries and/or

beverages to determine iron and zinc bioaccessibilities, and protein quality, respectively. Phenolics were extracted from slurries and beverages using acidified methanol [1% (v/v) conc. HCl in methanol] and the total phenolic content [TPC, Folin-Ciocalteu (F-C) method] and antioxidant properties of extracts against 2,2´-azinobis [3 ethylbenzothiazoline-6-sulphonic acid] (ABTS) diammonium salt were determined.

MALDI-TOF MS revealed the dominance of *Weissella confusa*, *Enterococcus casseliflavus*, *E. hermanniensis*, *Lactobacillus acidipiscis*, *L. salivarius*, *L. lactis*, *L. fructivorans*, and *L. lactis* ssp. *tructae* at the end of finger millet fermentations. 16S rRNA sequencing revealed the dominance of *Weissella* at the end of fermentation followed by the genera *Enterococcus* and *Lactococcus* only in the formulation with added maize starch. Overall, there were no differences in microbial diversity and relative abundance within the fermentation groups.

Starch source did not affect IVPD and reactive lysine content of beverages. Lactic acid fermentation improved IVPD with fermented and cooked samples showing higher IVPD than unfermented and cooked samples. This accentuates the role of fermentation in alleviating the negative effects of cooking on protein quality. The improvement in IVPD was attributed to pre-digestion of storage proteins and release of bound proteins at low pH which enhanced their availability to pepsin attack.

Iron and zinc bioaccessibility were improved by the addition of exogenous starch. The improvement was attributed to complexation of starch hydroxyl groups to mineral ions whose release during digestion enhanced mineral bioaccessibility *in vitro*. Although fermentation reduced the phytate content of the samples, no improvement was observed in bioaccessible iron, however, zinc bioaccessibility was improved relative to unfermented samples. The reduction in phytate content was attributed to dephosphorylation of phytate by endogenous phytases activated at low pH. The reduction in iron bioaccessibility can be due to iron chelation by non-phytate organic compounds such as phenolic compounds.

The addition of starch improved TPC and Antioxidant Activity (AA) of finger millet-based beverages. The increase was attributed to improved fermentation efficiency and better mobilisation of hydrolytic enzymes, which presumably helped release of bound phenolics. Although cooking did not affect TPC, AA was reduced. The decrease in AA suggested the

presence of heat unstable antioxidants whose destruction during heat processing led to a decrease in AA of beverages.

Lactic acid fermentation, with or without added exogenous starch, shows the potential to improve zinc bioaccessibility, protein quality (IVPD), and antioxidant properties of finger-millet beverages. Soured finger millet-based foods can potentially be used to reduce mineral and protein malnutrition, and oxidative stress-related NCDs in SSA.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS

AA	Antioxidant activity
ABTS	2,2´-azinobis [3 ethylbenzothiazoline-6-sulphoric acid]
ANCOM	Analysis of composition of microbiome
ArA	Arachidonic acid
Br	Birefringence
CVDs	Cardiovascular diseases
DALYs	Disability-adjusted life years
DSG	Disintegrated starch granule
CE	Catechin equivalent
cfu	Coliform forming units
F-C	Folin-Ciocalteu
IDA	Iron deficiency anaemia
IVPD	*In vitro* protein digestibility
LAB	Lactic acid bacteria
MALDI-TOF MS	Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry
MRPs	Maillard reaction products
NCDs	Non-communicable diseases
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
OTUs	Operational taxonomic units
-OH	Hydroxyl group
PERMANOVA	Permutational multivariate analysis of variance
QIIME	Quantitative Insights into Microbial Ecology
ROS	Reactive oxygen species
rRNA	ribosomal ribonucleic acid
SB	Stained block-let
SDS	Stained disintegrated starch
SSA	Sub-Saharan Africa
TPC	Total phenolic content
TTA	Titratable acidity

CHAPTER 1: INTRODUCTION AND PROBLEM STATEMENT

The crucial role of cereals in the food and nutrition security of resource-constrained communities of Sub Saharan Africa (SSA) cannot be overemphasized (Phiri, Schoustra, van den Heuvel, Smid, Shindano & Linnemann, 2019; Raheem, Dayoub, Birech & Nakiyemba, 2021). Among the different cereal grains cultivated for human consumption, maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.) and finger millet (*Eleusine coracana*) are most widely cultivated hence contribute substantially to the total dietary nutrient intake of low-income groups in Africa (Edmonds, Abreu, West, Caasi, Conley, Daft, Desta, England, Farris & Nobles, 2009; Gabaza, Shumoy, Louwagie, Muchuweti, Vandamme, Du Laing & Raes, 2018a). According to Adelakun and Oyelade (2011), cereal-based products contribute approximately 77% of the total energy intake in Africa, while total iron and zinc dietary intake provided by cereals is estimated at 57% and 56%, respectively (Joy, Ander, Young, Black, Watts, Chilimba, Chilima, Siyame, Kalimbira & Hurst, 2014).

Cereal grains are rich in nutrient inhibitors like phytate, polyphenols, and dietary fibre, which bind to nutrients such as proteins and minerals reducing their bioavailability in the human body (Nkhata, Ayua, Kamau & Shingiro, 2018). It is therefore inferred that the extensive consumption of monotonous cereal-based diets is one of the causes of nutrient deficiencies, hence the prevalence of malnutrition in SSA (Baye, Guyot, Icard-Vernière, Rochette & Mouquet-Rivier, 2015; Gabaza *et al.*, 2018a). Despite their antinutritional properties, bioactive phenolic compounds confer health-promoting antioxidant properties in food, therefore, play an essential role in the prevention of non-communicable diseases (NCDs), such as cancers and cardiovascular diseases (CVDs) by counteracting oxidative stress and reducing free radical cellular damage (Adebo & Gabriela Medina-Meza, 2020). Processing methods that enhance the bioavailability of nutrients and health-promoting constituents, particularly antioxidants, are most favourable.

The utilization of cereal grains for food in SSA mostly involves spontaneous fermentation into beverages, porridges, or dumplings such as *mahewu*, *gowe*, *ting*, *togwa*, *kenkey*, *mawe*, and many others (Phiri *et al.*, 2019). Due to the nutritional and health benefits linked to fermentation, increased consumption of fermented cereal-based products may help alleviate problems of nutrient malnutrition and NCDs experienced in SSA. These fermentation benefits are a result of microbial biochemical activities within the food matrix. In a study by Adebo,

Njobeh and Kayitesi (2018), an improvement in the antioxidant capacity of whole-grain *ting* (fermented sorghum beverage) fermented by *L. fermentum* strains was reported. The flavonoids and phenolic acid levels were slightly increased in the samples and it was suggested that hydrolytic enzymes from LAB strains may have catalysed the hydrolysis of bound phenolics into biologically active simple and soluble-free forms. Adeyanju, Kruger, Taylor and Duodu (2019) also reported higher bioaccessible iron and zinc in fermented sorghum-based beverages compared to unfermented samples. In the same study, the increase in bioaccessible iron and zinc corresponded with a reduction in phytate content. It was suggested that the acidic environment, created by microflora through starch hydrolysis, provided favourable conditions for hydrolysis of phytate by endogenous phytase reducing its ability to chelate divalent ions.

The co-fermentation of different cereals during the processing of cereal-based products is a common practice. For example, during fermentation of finger millet to make traditional beverages such as *ambali* (from India) and *mahewu* (from Southern Africa), rice starch slurry or maize flour and sorghum or wheat are added, respectively. While the fermentation properties and benefits of finger millet are known (Antony & Chandra, 1997; Antony & Chandra, 1998; Antony & Chandra, 1999; Makokha, Oniang'o, Njoroge & Kamar, 2002; Gabaza, Shumoy, Muchuweti, Vandamme & Raes, 2016; Gabaza *et al.*, 2018a), there is scanty information in literature on how addition of exogenous starch sources during finger millet fermentation affects the microbial dynamics of slurries, as well as, the nutritional and health benefits of such products. Knowledge of the microbial diversity of co-fermented finger millet-based products will inform the selection of starter cultures for the production of safe and standardised products with enhanced nutrient and health-promoting properties. This study investigates the effect of adding an exogenous source of starch from rice and maize during production of a lactic acid fermented finger millet-based beverage on the microbial, nutritional and bioactive properties of the beverages with the aim of contributing towards improving the nutritional and health status of low and middle-income groups of SSA.

Included in this dissertation is an in-depth review of literature relevant to this study, that is, the burden of malnutrition and NCDs in Africa, factors contributing to the poor nutrient quality of cereal-based diets, health and fermentation benefits of finger millet and microbiome involved in the fermentation process. After the literature review are the hypotheses and study objectives followed by the research chapter which is divided into two parts: microbiological diversity of finger millet-based slurries, and nutritional and antioxidant properties of finger millet

beverages as influenced by starch source during lactic acid fermentation. The general discussion evaluates the research methodology and presents an in-depth discussion of research findings. Finally, are the conclusions and recommendations of the study.

CHAPTER 2: LITERATURE REVIEW

2.1 Burden of macronutrient and micronutrient deficiencies in Africa

Macronutrient and micronutrient deficiencies are common in developing countries and are linked to several factors, including inadequate food, poor quality diets, and poor bioavailability and digestibility of nutrients. Poor nutrient bioavailability and digestibility are often associated with high amounts of nutrient inhibitors, especially in plant foods, such as whole-grain cereals, which are known to be high in phytate and tannins. Of significant public health concern are deficiencies of iron, zinc, folate, and protein since they are involved in a broad range of biochemical processes in the body at different stages of life (Ramakrishnan, 2002; Hagmeyer, Haderspeck & Grabrucker, 2015).

Iron deficiency has long been considered the major form of malnutrition due to the far-reaching consequences associated with it. It is considered to contribute to child, maternal and perinatal deaths as well as impaired cognitive and motor development, poor physical development, and ineffective immune response (Thompson, 2007; Muthayya, Rah, Sugimoto, Roos, Kraemer & Black, 2013). Worth noting is the fact that the existence of iron deficiency can take two forms, that is, with anaemia or without anaemia. Of these two forms, iron deficiency associated with anaemia is most severe (WHO/UNICEF/UNU, 2001). In 2000, 841,000 deaths and 35,057,000 disability-adjusted life years (DALYs) globally were attributed to Iron-deficiency anaemia (IDA). In its distribution, IDA is highly concentrated in Africa, where 62.3% of the population is affected while 3.6% of children between the ages 6 months to 5 years are struggling with severe anaemia (Stoltzfus, 2001; Stoltzfus, 2003; WHO, 2015). It is also estimated that Africa has the highest iron deficiency anaemia prevalence rates in women where 55.8% and 44.4% of women (pregnant and non-pregnant, respectively) are affected (De Benoist, Cogswell, Egli & McLean, 2008).

On the other hand, zinc deficiency is also causing serious health consequences in Africa, where it is estimated to account for over nine million DALYs (Walker, Ezzati & Black, 2009). Generally, information regarding zinc deficiency is limited, which tends to hinder interventions channelled towards suppressing the problem. This limitation can be due to the limited number of valid zinc biomarkers and the high costs of assessing zinc adequacy compared to other minerals. In a study by Wessells and Brown (2012), approximately 17.3% of the global population was at risk of zinc deficiency with the highest prevalence estimated in SSA, where

the country-specific prevalence of inadequate zinc intake is either greater than 25% or falls between 15 and 25% (Wessells & Brown, 2012).

In Africa, the intake of dietary proteins is generally lower than the World Health Organization (WHO) recommended intake of 0.66 g protein/kg body weight per day (Schönfeldt & Hall, 2012). It is on that note that the protein intake, as well as its bioavailability and digestibility are under scrutiny in the various foods consumed in Africa, especially cereal-based products. One of the contributing factors to protein deficiency in developing countries is a heavy reliance on cereals as major sources of protein (Schönfeldt & Hall, 2012). Several reviews attest that cereals are rich sources of complex antinutritional compounds that bind and entrap nutrients limiting their release from the food matrices (Blandino, Al-Aseeri, Pandiella, Cantero & Webb, 2003; Gabaza, Muchuweti, Vandamme & Raes, 2017; Nkhata *et al.*, 2018).

2.2 Burden of diet-related NCDs in Africa

In low- and middle-income regions of the world, NCDs are still major public health concerns. These include diabetes, cardiovascular diseases, cancer, and chronic lung diseases (Ezzati, Pearson-Stuttard, Bennett & Mathers, 2018; Niessen, Mohan, Akuoku, Mirelman, Ahmed, Koehlmoos, Trujillo, Khan & Peters, 2018). ln the year 2016, 71% of 56.9 million deaths were attributed to NCDs, thus, accounting for over half of the overall global disease burden (Bennett, Stevens, Mathers, Bonita, Rehm, Kruk, Riley, Dain, Kengne & Chalkidou, 2018). Africa, especially Sub Saharan countries, is still battling with the sharp rise in NCDs undermining efforts towards the manifestation of Agenda 2063, unless urgent measures are adopted (Owino, 2019). Although considerable measures have been implemented to address the NCD pandemic, Africa has not managed to maintain its pace hence the unmet international commitments, including the NCD global action plan 2013-2020 led by World Health Organization (WHO) (Nyaaba, Stronks, Aikins, Kengne & Agyemang, 2017). Different approaches and strategies to control and minimise new cases of the problem have been proposed. These include addressing diet-related risk factors, which is a promising approach seeing that it can be applied even in communities where resources are limited by promoting the utilization of locally available cereal grains. Such practical interventions are paramount towards the achievement of the Sustainable Development Goal (SDG) target 3.4, which aims to reduce mortalities from NCDs by one third by the year 2030 (Bennett *et al.*, 2018).

A high prevalence of NCDs has been reported since the political transition in South Africa (Alberts, Urdal, Steyn, Stensvold, Tverdal, Nel & Steyn, 2005). Efforts channelled towards combating the trend have been marginalized due to the overwhelming burden from communicable diseases particularly HIV/AIDS and Tuberculosis (TB), which have received considerable attention over the years (Mayosi, Flisher, Lalloo, Sitas, Tollman & Bradshaw, 2009). This explains the observation that the age-standardized death rate from NCDs in South Africa is currently higher than that of HIV/AIDS and TB combined, of which CVDs rank the highest (Schutte, 2019). Population-based studies done in South Africa have revealed the disproportionality in the distribution of NCDs where the highest disease burden has been reported in low-income populations, predominantly rural communities (Schutte, 2019).

A common risk that has been identified so far for these diseases is unhealthy food choices, especially by the adult South African population who are mostly affected. The national prevalence data has shown a considerable shift in dietary patterns, especially among black people who account for over three-quarters of the South African population (Bourne, Lambert & Steyn, 2002; Norman, Bradshaw, Schneider, Joubert, Groenewald, Lewin, Steyn, Vos, Laubscher & Nannan, 2007; Department of Health, 2007). The disproportional distribution of the disease burden in South Africa highlights the need to scale up measures towards the management of risk factors as well as the application of cost-effective measures for the prevention of severe damage from diseases (Mayosi *et al.*, 2009) Some of the policy interventions by the South African government to reduce the national NCDs burden include advocative legislative plans that regulate the consumption of processed foods while promoting healthier traditional diets. This is achieved by taxation of undesirable processed foods while healthier choices are exempted from taxation (Spires, Delobelle, Sanders, Puoane, Hoelzel & Swart, 2016).

2.3 Finger millet: Structure, production and food uses

Finger millet (*E. coracana* L.*)* is an important cereal crop in the arid and semi-arid regions of the world where it is cultivated mainly for human consumption (Dayakar Rao, Bhaskarachary, Arlene Christina, Sudha Devi, Vilas & Tonapi, 2017). Apart from its importance due to superior nutritional properties over other cereals, it is viewed as an agronomically sustainable crop due to its ability to maintain optimum yield under drought conditions with low cultivation/production inputs (Shobana, Krishnaswamy, Sudha, Malleshi, Anjana, Palaniappan & Mohan, 2013; Kumar, Metwal, Kaur, Gupta, Puranik, Singh, Singh, Gupta, Babu & Sood,

2016a). Different varieties of finger millet exist, which are differentiated based on grain colour (red, brown, light brown, and white) (Devi, Vijayabharathi, Sathyabama, Malleshi & Priyadarisini, 2014; Ramashia, Anyasi, Gwata, Meddows-Taylor & Jideani, 2019).

Morphologically, finger millet is a small, globular kernel of diameter 1.0-1.5 mm (Siwela, 2009). Like other cereals, it is composed of three main anatomical features, that is the seed coat (testa), germ, and the endosperm (Figure 2.1). However, its kernel structure is different in that it is utricular meaning that the pericarp is only attached to the seed coat at one point (Hoseney, 1994; Ramashia *et al.*, 2019). The finger millet seed coat has five distinct layers which make it unique from other millets such as pearl millet, proso millet, and foxtail millet (FAO, 1995 in Shobana *et al*., 2013). The seed coat is bound tightly to the aleurone layer and the starchy endosperm. The starchy endosperm has two regions, that is, the corneous (glassy) and floury endosperm (Figure 2.1). It is within the corneous endosperm that starch granules are highly organised as compared to the floury endosperm where the starch granules are loosely packed (McDonough, Rooney & Earp, 1986). The size of starch granules in the starchy regions varies from 3 to 21 µm but generally, the starchy granules in the corneous region are bigger hence more prone to enzymic hydrolysis (FAO, 1995 in Shobana *et al*., 2013).

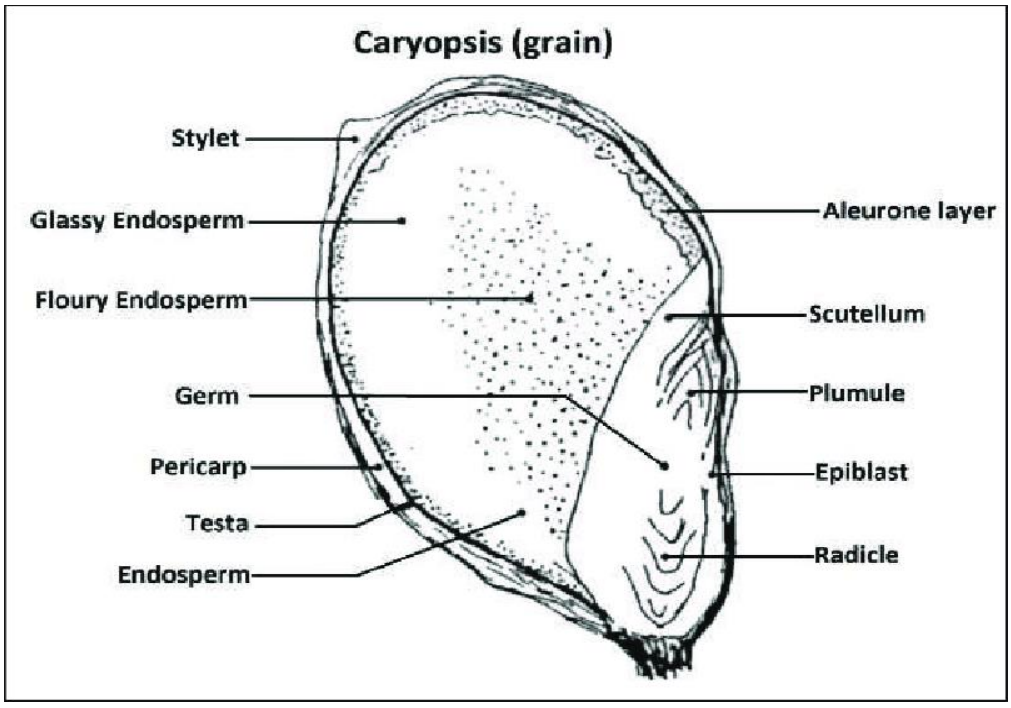


Figure 2.1. Finger millet grain structure (Dayakar Rao, Bhaskarachary, Arlene Christina, Sudha Devi, Vilas & Tonapi, 2017)

India and Africa are the powerhouses of finger millet production in the world. In India, Karnataka State has been ranked as the leading producer of finger millet accounting for 58% of the total finger millet production in India (Chandra, Chandra & Sharma, 2016). Globally, at least 4.5-5 million tonnes of finger millet are produced annually of which 2 million tonnes are produced in Africa where it is widely cultivated in Ethiopia, Kenya, Nigeria, Malawi, Mozambique, South Africa, Tanzania, Uganda, Zambia, and Zimbabwe (Ramashia *et al.*, 2019). This leaves India as the largest producer of finger millet, contributing approximately 60% of the total global production (Gull, Jan, Nayik, Prasad & Kumar, 2014). In Africa, Nigeria has been the leading producer of finger millet since 2010 with an estimated annual yield of up to 1 384 900 tonnes in 2014 (Ramashia *et al.*, 2019).

Finger millet can be processed into different traditional and commercial products with the primary aim of transforming the grains into edible value-added convenience foods (Ramashia *et al.*, 2019; Rathore, Singh, Kamble, Upadhyay & Thangalakshmi, 2019). Some of the common and widely used processes include decortication, soaking, malting, milling, fermentation, cooking, popping, roasting, radiation, extrusion as well as compositing it with other cereal or legume flours to improve nutritional and functional properties of the end products (Saleh, Zhang, Chen & Shen, 2013; Ramashia *et al.*, 2019).

In Indian cuisine, finger millet is often milled and the wholemeal flour used in the production of unleavened flatbreads (*roti*), finger millet balls, noodles, soups, dumplings (*mudde*), and thin porridges. In addition to these, it can be processed into popped, malted, and fermented products such as fermented steamed cakes (*idli*), fermented pancake (*dosa*), and beverages, including ambali (Shobana, Sreerama & Malleshi, 2009; Shobana *et al.*, 2013). In Africa, most finger millet-based foods are fermented into thin porridges or gruels and used as important staple foods for adults and weaning foods for infants, for example, *ting* and *mahewu* (Gabaza *et al.*, 2017). There is increasing use of a combination of traditional and modern techniques to produce finger millet-based food products with enhanced nutritional and health-promoting properties. Such products play a crucial role in contributing to the improved nutritional and health status of consumers, especially the low- and middle-income populations in developed countries where cereals are the main source of nutrients.

2.4 Factors affecting protein quality and mineral bioaccessibility of cereals

Whole grain cereals are one of the most important sources of food worldwide, with the potential to contribute significantly to the dietary protein and mineral intake (Saleh *et al.*, 2013; Oghbaei & Prakash, 2016). Despite this, whole grain cereals are sometimes inferior in nutritional quality parameters such as protein quality and mineral bioaccessibility when compared to other non-cereal foodstuffs due to abundance of antinutritional factors (ANFs), particularly phytate, tannins, and dietary fibre (Blandino *et al.*, 2003; Gilani, Xiao & Cockell, 2012). These compounds adversely affect the digestibility of protein fractions, the bioavailability of indispensable amino acids as well as the bioaccessibility of minerals by forming insoluble complexes with these essential nutrients (Gilani *et al.*, 2012).

2.4.1 Phytate

Phytate also known as *myo*- inositol hexaphosphate (IP6) or phytic acid is a naturally occurring heat-stable compound found in cereal grains and other plant food sources where it serves as an important source of phosphate and inositol during plant germination (Lopez, Leenhardt, Coudray & Remesy, 2002; Gilani *et al.*, 2012). It is a hexaphosphoric ester of hexahydric cyclic alcohol meso-inositol (Kumar, Sinha, Makkar & Becker, 2010) hence the chemical name assigned to it. In plant tissues, phytic acid exists as a salt (phytate) of mono- and divalent cations K^{+}, $Mg^{2+,}$ and Ca^{2+} (Kumar *et al.*, 2010). It is evenly distributed within the protein bodies of dicotyledonous seeds and in the protein-rich aleurone layers of monocotyledonous seeds, therefore, processing treatments such as polishing may reduce the proportion of phytate in the finished product (Ravindran, Cabahug, Ravindran & Bryden, 1999; Gilani *et al.*, 2012).

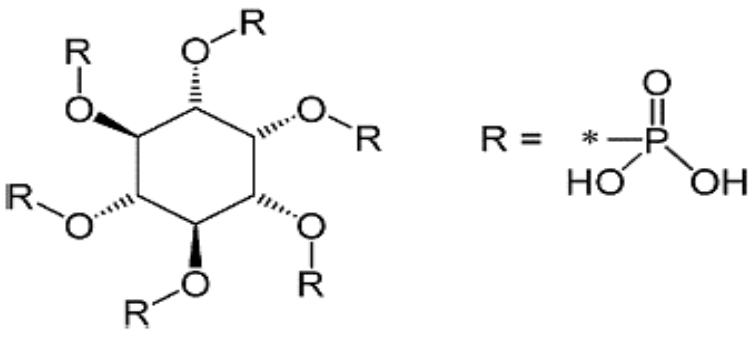


Figure 2.2. Chemical structure of phytate (Kumar, Sinha, Makkar & Becker, 2010)

Figure 2.2 shows the unique chemical structure of phytate which is responsible for its characteristic properties. The phytate molecule is abundant in highly charged phosphate groups, it is for this reason that it can easily chelate and bind with divalent mineral nutrients such as calcium, magnesium, zinc, and iron in plant tissues (Ravindran *et al.*, 1999; Reddy & Sathe, 2001). Once the minerals are bound, they lose their ionic state, hence cannot bind to the carrier proteins for transport across the intestinal cell membrane. As a result, there is a significant reduction in the overall bioaccessibility of minerals in the human gastrointestinal (GI) tract (Lopez *et al.*, 2002).

Due to the ionic nature of phytate, it can also react directly or indirectly with protein structures under favourable pH conditions. At low pH, phytate phosphoric acid groups directly interact with charged proteins through electrostatic linkages with cationic groups of amino acid residues such as arginine, lysine, and histidine. When the charge on the protein is neutralized, for example in the small intestines due to the high pH, phytate can no longer bind to the protein directly, however, complexes between phytate and protein can still form indirectly by multivalent bridging involving cations such as calcium (Ravindran *et al.*, 1999; Selle, Ravindran, Caldwell & Bryden, 2000; Kumar *et al.*, 2010). Both the phytate: protein and phytate: mineral: protein complexes are insoluble hence interfere adversely with the functionality of proteins and cations in the GI tract. Research has also suggested that phytate can also indirectly reduce protein digestibility and quality *in vitro* by binding with proteolytic enzymes (Ravindran *et al.*, 1999; Kumar *et al.*, 2010).

Different food processing and preparation techniques have been used to reduce the level of phytate in cereal grains. Successes reported so far have been based on the use of the following techniques, individually or in combination: soaking, germination, cooking, fermentation, roasting, microwaving, and many others (Reddy & Sathe, 2001). The use of exogenous phytases isolated from plant sources, micro-organisms, and animals has been another effective alternative to food processing (Kumar *et al.*, 2010).

In a report by Antony and Chandra (1999), lactic acid fermentation of two finger millet varieties (red and white) decreased the phytate content by 23-26%, whilst improving mineral availability and *in vitro* protein digestibility by 5.5-22%. Similar observations were reported for pearl millet where a gradual increase in protein digestibility of up to 51% was observed with an increase in fermentation temperature and time (Dhankher & Chauhan, 1987). This can, in part, be

attributed to the activation of endogenous phytases at low pH leading to the dissociation of phytate: protein complexes that hamper protein solubility and digestibility (Kumar *et al.*, 2010).

2.4.2 Tannins

Grain varieties of cereals such as dark finger millet, sorghum and barley contain considerable levels of condensed tannins (Dykes & Rooney, 2007). These are complex high molecular weight polyphenolic compounds often implicated for reducing the nutritional quality of cereal grains (Dykes & Rooney, 2007; Gilani *et al.*, 2012). Condensed tannins (also known as proanthocyanidins, Figure 2.3) are polymerised products of flavan-3-ols (e.g. catechin and epicatechin, Figure 2.4) and/or flavan-3,4-diols of which flavan-3-ol-based condensed tannins are widely studied (Hagerman, 2002) possibly due to their abundance.

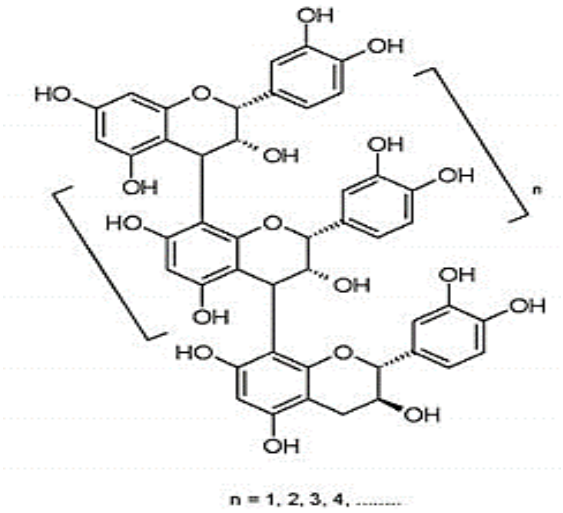


Figure 2.3. Chemical structure of condensed tannins (proanthocyanidins) found in cereal grains (Dykes & Rooney, 2007)

The interaction between tannins and proteins depends on several factors including pH of the medium and abundance of hydroxyl groups (-OH) in the structure of the tannin molecule (Figure 2.3 and Figure 2.4). In general, tannins can interact with proteins through hydrophobic interactions, hydrogen bonding, which is mediated by the abundant hydroxyl groups, and to an extent, ionic bonding. Involved also, are covalent cross-linkages when tannins are oxidised to highly reactive intermediates, ortho-quinones (Lamy, Pinheiro, Rodrigues, Capela-Silva, Lopes, Tavares & Gaspar, 2016). Such interactions between tannins and protein negatively impact protein digestibility and availability of amino acids thus reduce the protein quality of

whole-grain cereals. Precisely, hydrophobic interactions involve the aromatic rings of the tannin molecule and the hydrophobic sites of the protein molecule, hydrogen bonds occur between the hydroxyl groups of tannins and the H-acceptor of the protein molecule and finally, ionic bonds are a result of the interaction between +charged groups of amino acids like lysine and the -OH groups of polyphenols (Le Bourvellec & Renard, 2012). Tannins have also demonstrated the ability to inhibit proteolytic enzyme activity through the formation of inactive enzyme-inhibitor (tannins) complex, enzyme-substrate-inhibitor complex, or substrate-inhibitor complex (Le Bourvellec & Renard, 2012).

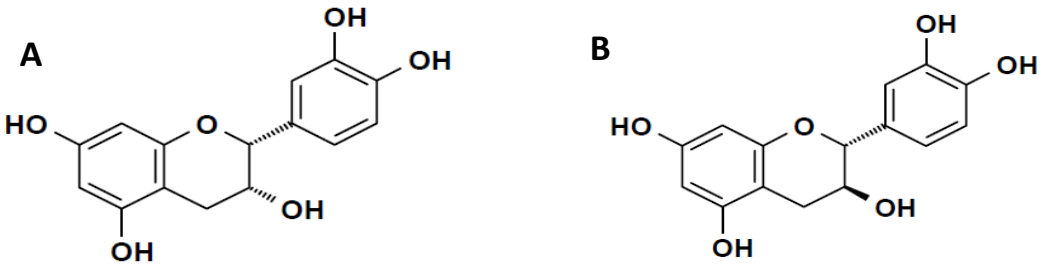


Figure 2.4. Structure of epicatechin (A) and catechin (B) (Hagerman, 2002)

The tannin OH groups are also chelation sites for metal ions such as iron hence an increase in the metal ion binding capacity of tannins with an increase in the number of -OH groups, is expected. Just like protein, the reaction is pH dependant, therefore, mixtures of mono- bis-type and sometimes tris-complexes are formed with iron within a pH range of 1 – 7 (Friman, Höglund, Högberg & Agnemo, 2004).

2.4.3 Dietary fibre

In addition to starch, whole grain cereals are also high in non-starch polysaccharides also known as dietary fibre. A more concise definition explains dietary fibre as edible carbohydrate polymers composed of ten or more monomer units, which cannot be hydrolysed by endogenous enzymes in the human small intestines (Rousseau, Kyomugasho, Celus, Hendrickx & Grauwet, 2020). These are divided into soluble and insoluble fibre based on their solubility in water as a function of pH. Insoluble fibre, such as cellulose, hemicellulose, lignin, and resistant starch is highly abundant in whole grain cereals (Figuerola, Hurtado, Estévez, Chiffelle & Asenjo, 2005; Rousseau *et al.*, 2020), including finger millet, sorghum, barley, and others.

The free carboxyl groups on some sugar residues, uronic acid, and hydroxyl groups, on the surface of cellulose have been reported to chelate and bind minerals as well as proteins forming insoluble matrixes which cannot be hydrolysed by human digestive enzymes (Rousseau *et al.*, 2020). The formation of complexes between dietary fibre and minerals is seen to be a result of either electrostatic forces, chelation, or ion exchange capacity. Although the bound minerals are hypothesised to be released in the large intestines following possible microbial fermentation, this has implications on the bioavailability of minerals since maximum absorption is in the small intestines (Rousseau *et al.*, 2020). Comparing soluble and insoluble dietary fibre, it has, however, been shown that soluble dietary fibre interacts strongly with metal ions, while insoluble dietary fibre shows a negligible capacity to do so (Nair, Asp, Nyman & Persson, 1987). Therefore, a conclusion can be drawn that insoluble dietary fibre, which is highly abundant in whole grain cereals, may have little effect on mineral bioaccessibility.

2.5 Phytochemical-related health-promoting properties of millets

Along with their nutritional properties, whole grain millets are high in phytochemicals, particularly phenolic compounds, although significant variations in quantity and type have been reported based on species, variety, and geographic distribution (Léder, 2004; Taylor & Duodu, 2015). In a study by Chandrasekara and Shahidi (2010), grain varieties of finger, kodo, proso, foxtail, little, and pearl millet were evaluated for their phenolic content. The total phenolic content (TPC) of soluble extracts for the different millet species ranged between 7.19 to 32.39 µmol of ferulic acid equivalent (FAE)/g of defatted meal. Varieties with a dark brown testa such as kodo and finger millet have been reported as having a higher TPC compared to varieties with a white or yellow testa such as pearl and proso millet (Chandrasekara & Shahidi, 2010). Due to their high antioxidant capacity, phenolic compounds are linked to several health-promoting properties, including reduced risk of non-communicable diseases (Dykes & Rooney, 2007; Zhu, 2015). Discussed below are some of the health-promoting properties of millets.

2.5.1 Antioxidant properties

During normal metabolic activity, the body can produce reactive oxygen species (ROS) such as superoxide [$O_2^{\bullet}$], hydroxyl [$OH^{\bullet}$], hydroperoxyl [$HOO^{\bullet}$] and peroxyl [$ROO^{\bullet}$] free radicals (Chiang, Wu, Yeh, Chu, Lin & Lee, 2006). ROS can easily induce molecular, cellular, and tissue pathogenesis causing chronic diseases such as CVDs, cancer, type II diabetes, heart diseases, and others. This is highly likely especially if ROS occur in excessive amounts so that

they overwhelm the body's antioxidant defense system, a phenomenon known as oxidative stress (Walter & Marchesan, 2011; Singh & Kimothi, 2018).

Millet phenolics have received considerable attention over the years due to their proven antioxidant properties owing to the functional hydroxyl groups attached to the aromatic rings in their basic structure (Issa, Volate & Wargovich, 2006). Different mechanisms have been proposed to explain how phenolic compounds exert their antioxidant potential, including chain-breaking mechanisms by directly scavenging active radicals or donating hydrogen atoms to the electron-deficient radicals to form less reactive radicals (Issa *et al.*, 2006; Lobo, Patil, Phatak & Chandra, 2010; Walter & Marchesan, 2011). The hydroxyl groups are also sites for chelation of pro-oxidant metals such as iron and copper maintaining their capacity as endogenous antioxidants (Friman *et al.*, 2004; Walter & Marchesan, 2011). Besides, phenolic compounds can also exert their antioxidant effects on biological systems by increasing the activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase or inducing the synthesis of antioxidant proteins which trigger the release of antioxidant enzymes (Chiang *et al.*, 2006; Lobo *et al.*, 2010).

Phenolic extracts of six millet varieties (kodo, finger, proso, pearl, little, foxtail, and little millet) were shown to present potential health benefits due to demonstrated *in vitro* Trolox equivalent antioxidant efficacy against ABTS radical cation. Variations in antioxidant capacity were attributed to differences in genotype and TPC of extracts wherein kodo millet showed a higher antioxidant capacity compared to the other millet grains which corresponded with its higher TPC reported in the same study (Chandrasekara & Shahidi, 2010). In a different study, finger millet (Ravi) phenolic extracts showed a dose-dependent chelating activity (IC_{50} value of 0.85 mg/mL) to ferrous ions, which contribute to DNA oxidation by generating hydroxyl radicals (Chandrasekara & Shahidi, 2011a). Finger millet contains flavan-3-ol monomers and dimers of procyanidin B1 and B2 which explains its higher ferrous ion chelating ability compared to other millets such as kodo and pearl millet (Chandrasekara & Shahidi, 2011a), although with noteworthy chelating activity as well.

The protective role of millet phenolic compounds as antioxidants has also been demonstrated in animal model studies. A diet with 55% whole grain finger and kodo millet for 28 days increased the activity of antioxidant enzymes catalase and glutathione reductase while significantly lowering blood sugar in alloxan-induced hyperglycemic rats (Hegde, Rajasekaran

& Chandra, 2005). Oxidative free radicals have been implicated in the degeneration of pancreatic *ß*-islet cells contributing to the development of diabetes mellitus. Therefore, radical scavengers such as millet phenolics may help protect animals against free radical-induced diabetes (Hegde *et al.*, 2005).

2.5.2 Anti-diabetic properties

One of the key approaches in the management of type II diabetes is decreasing blood glucose levels by delaying the rate at which glucose is metabolised in the body postprandial. Polyphenols such as condensed tannins found in finger millet have shown to be effective inhibitors of enzymes α-glucosidase and pancreatic amylases involved in carbohydrate metabolism, delaying the digestion of starch and release of glucose into the bloodstream (Kumar *et al.*, 2016a). The low glycemic response of diets based on finger millet has been widely reported (Kumari & Sumathi, 2002; Shobana *et al.*, 2009; Chandra *et al.*, 2016). This can be accurately linked to the ability of phenolic compounds to bind to enzyme reactive sites altering their catalytic activity (Kunyanga, Imungi, Okoth, Biesalski & Vadivel, 2012).

In a study by Shobana *et al.* (2009), phenolic extracts from millet seed coat showed a strong non-competitive inhibition towards α-glucosidases and pancreatic amylases at IC_{50} values of 16.9 and 23.5 µg, respectively, emphasizing the therapeutic potential of millet-based diets in the management of type II diabetes. Polyphenol-starch interactions also regulate glucose levels in biological systems by decreasing *in vitro* starch digestibility. This can be attributed to the reduced susceptibility of complexed starch to hydrolysing enzymes, hence the low glycemic index of cooked millet-based diets (Zhu, 2015).

2.5.3 Anti-inflammatory properties

During an injury or attack by pathogens, the body's inflammatory response is triggered. Different enzyme-controlled inflammation pathways exist such as cyclooxygenase (COX), lipoxygenase (LOX), and phospholipase A2 (PLA2) pathways involving the metabolism of arachidonic acid (ArA) to form prostaglandins (PGs), hydroperoxyeicosateraenoicacids (HETEs) or leukotrienes (LTs), which play a key role in inflammation (Issa *et al.*, 2006). Involved also are cytokine, nitric oxide synthase (NOS), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), though classified as ArA independent mediators (Issa *et al.*, 2006). Although not a disease in itself, there are instances where the body fails to control

inflammation leading to tissue damage and disruption of cell signaling pathways, thereby triggering other diseases such as colorectal cancer (Serhan & Savill, 2005).

Flavonoids, also found in millet, have demonstrated their functionality as therapeutic agents in animal model studies owing to their ability to inhibit the activity of enzyme isoforms involved in the synthesis of ArA yielding inflammatory mediators (Lindahl & Tagesson, 1993; Kim, Pham & Ziboh, 2001; Issa *et al.*, 2006) and their nitric oxide radical scavenging capacity (Taylor, Belton, Beta & Duodu, 2014). Several flavonoids have been reported in millet, for example, flavones in pearl millet, apigenin and luteolin in fonio millet, and luteolin, tricin, and N-(p-coumaroyl) serotonin in Japanese barnyard millet. Serotonin was shown to exhibit *in vitro* anti-inflammatory properties by inhibiting the synthesis of cytokine from human monocytes, an effect based on indirect suppression of cytokine messenger ribonucleic acid [mRNA] expression by inhibiting the activation of NF-kB (Kawashima, Hayashi, Takii, Kimura, Zhang, Nagatsu, Sakakibara, Murata, Oomoto & Onozaki, 1998; Watanabe, 1999; Shahidi & Chandrasekara, 2013). Foxtail millet was also reported as having the ability to reduce inflammation indicator C- reactive protein in hyperlipidaemic rats which was linked to reduced risk of CVDs (Lee, Chung, Cha & Park, 2010).

Shi, Shan, Li, Song and Li (2017) investigated the effects of foxtail phenolics on the pro-inflammatory cytokines induced by lipopolysaccharide (LPS) in human colorectal adenocarcinoma cell line (HT-29 cells) and nude mice. They reported that inner shell-bound polyphenols from foxtail millet can display anti-inflammatory effects *in vivo* and *in vitro*. Foxtail millet bran phenolics were found to reduce the level of pro-inflammatory cytokines in a dose-dependent manner while promoting the expression of anti-inflammatory cytokine IL-10 through the blockage NF-kB nuclear translocation. Although the mechanisms behind polyphenols and anti-inflammatory properties are still scantily understood, based on these results, it can be concluded that millet phenols are important anti-inflammatory compounds whose understanding can help combat the escalating incidences of diet-related non-communicable diseases.

2.5.4 Anti-cancer properties

Some of the widely researched bioactive compounds with potential anti-cancer properties naturally found in plants include phenolics with a demonstrated ability to terminate free radicals, singlet oxygen species, or other ROS implicated in cellular oxidation and increased

risk of carcinogenesis (Kumar *et al.*, 2016a). Millet phenolic extracts are effective, at varying degrees, in the prevention of cancer initiation and progression *in vitro* due to their antioxidant properties (Chandrasekara & Shahidi, 2011a). Oxidative stress is one of the key contributors to cellular damage and pathogenesis of cancer, therefore, the regulation of oxidative stress by quenching ROS or inhibiting ROS-producing enzymes are ways in which phenolic compounds exert their beneficial effects (Chandrasekara & Shahidi, 2011a).

Xanthine oxidase is an enzyme that is known for generating ROS in the process of converting hypoxanthine to xanthine or in reperfusion injuries of the heart and small intestines. The relationship between cellular damage, cancer pathogenesis, and ROS makes xanthine oxidase an important enzyme in cancer initiation (Zhang, Blake, Stevens, Kanuler, Ward, Symons, Benboubetra & Harrison, 1998; Lin, Chen, Ho & Lin-Shiau, 2000). Millet phenolic extracts at a concentration of 1 mg/mL inhibited the activity of xanthine oxidase *in vitro*. Among the different millet varieties used in the study, kodo millet had the highest xanthine oxidase inhibitory activity of 69%. The enzyme inhibitory activity of millet phenolics was directly linked to the ferulic acid content of extracts which explains why kodo millet, which is generally high in trans-ferulic acid, had the highest activity (Chandrasekara & Shahidi, 2011a; Chandrasekara & Shahidi, 2011b).

In a similar study by Chandrasekara and Shahidi (2011a), finger millet and kodo phenolic extracts displayed 97% chemo-preventive properties against peroxyl radical-induced DNA scission due to their high flavonoids content compared to other millet varieties used in the study. The permanent damage of DNA due to oxidase stress is seen as the first step in cancer development (Valko, Izakovic, Mazur, Rhodes & Telser, 2004). In contrast, foxtail, proso, and little millet extracts seemed more effective in the inhibition of DNA scission induced by hydroxyl radicals than peroxyl radicals. The millet phenolic extracts were further investigated for their antiproliferative properties against HT-29 human colon adenocarcinoma cells where they exhibited anti-proliferative activity of up to 40% after 96 h of incubation (Chandrasekara & Shahidi, 2011a). Foxtail millet phenolic acids also showed a dose-dependent anti-proliferative activity on the growth of human breast cancer cells (MDA) and human HepG2 liver cancer cells (Zhang & Liu, 2015). Both results suggest that millet phenolics can effectively regulate both initiation and progression stages of cancer, hence are important health-promoting bioactive compounds.

2.5.5 Hypolipidemic properties and CVD prevention

Research evidence shows the link between dyslipidemia and the increased risk of CVD, which has been reported as one of the leading causes of death in different parts of the world (Bora, Das, Mohan & Barthakur, 2018). Some of the CVD risk factors include elevated levels of low-density lipoprotein cholesterol (LDL), reduced high-density lipoprotein cholesterol (HDL) levels, and increased oxidation of LDL (Lee *et al.*, 2010). Regular consumption of millet-based food products can help prevent CVDs due to the high levels of flavonoid-type phenolics in millet grains (Mellen, Walsh & Herrington, 2008; Taylor, 2017). Some of the millet grains with potential hypolipidemic benefits include pearl, proso, barnyard, finger, foxtail, and kodo (Lee *et al.*, 2010; Dayakar Rao *et al.*, 2017; Bora *et al.*, 2018).

Bora *et al.* (2018) conducted a study to evaluate the effects of diets supplemented with proso millet flour at 10, 20, and 40% on high-fat diet-induced hyperlipidemia rats for 28 days. Hyperlipidemia was induced by supplementing normal control rat diet with 20% coconut oil. Rats fed a high lipid diet showed a decrease in HDL while rats fed a high-fat diet supplemented with proso millet flour showed an increase in HDL. In the same study, diets supplemented with proso millet flour were also shown to decrease the total cholesterol levels in hyperlipidemia rats significantly. Similarly, Lee *et al.* (2010) compared the hypolipidemia properties of whole-grain foxtail millet, proso millet, and sorghum compared with white rice in hyperlipidemia rats for 5 weeks. At the end of the study, rats fed a diet supplemented with millet showed lower plasma triglycerides and total cholesterol levels as opposed to rats fed sorghum and rice.

2.6 Fermented finger millet products

Fermentation is an ancient and widely practised technique that is used to preserve and transform food into products of variable flavours and consistency. In some parts of the world, it is still an artisanal technique that is performed with limited understanding of the role of microorganisms involved in the biochemical process (Blandino *et al.*, 2003). The growth of microorganisms and the succession of microbial communities involved during fermentation play a crucial role in the development of the characteristic properties of the final product. This is because the transformation of the food matrix into various secondary and primary metabolites depends on the hydrolytic power of microbial enzymes which are secreted by a consortium of microbes during the fermentation process (Li, Chai, Li, Huang, Luo, Xiao & Liu, 2018).

The changes in the concentration, availability or digestibility of chemical compounds, which contribute to the nutritional attributes of fermented products, and their association with fermentation microbiota has been investigated. In a study by Ogodo, Ugbogu, Onyeagba and Okereke (2019), the effectiveness of LAB species (*Lactobacillus plantarum, L. rhamnosus, L. nantensis, L. fermentum, L. reuteri, Pediococcus acidilactici* and *L. brevis*) in improving the nutritional quality of sorghum flour was demonstrated. The improvement in protein digestibility was linked to secretion of proteolytic enzymes by LAB species during fermentation. The decrease in pH due to the production of lactic acid presumably enhanced the activity of proteolytic enzymes, which may have led to the partial breakdown of storage proteins into short-chain peptides and amino acids that can be easily digested (Mohiedeen, Tinay, Elkhalya, Babiker & Mallasiy, 2010; Alka, Neelam & Shruti, 2012).

In some studies, microbial community succession has been studied by isolating and identifying microbial diversity during the fermentation process through culture-dependent techniques or culture-independent techniques such as MALDI-TOF MS and high throughput Illumina MiSeq sequencing, respectively, and then linking microbial diversity with chemical properties of the final product (Li *et al.*, 2018; Resende, Pinheiro, Miguel, Ramos, Vilela & Schwan, 2018). Integrating microbial and biochemical dynamics in the study of fermented products has improved the understanding of the role of microorganisms in enhancing the health-promoting properties of fermented food as well as the selection of appropriate starter cultures to improve the efficiency of the fermentation process, ultimately the quality of the final product (Chakravorty, Bhattacharya, Chatzinotas, Chakraborty, Bhattacharya & Gachhui, 2016; Vilela, 2019). For example, the use of *L. fermentum* strains to improve acidification, sensory and antioxidant properties of *ting*, a fermented whole grain sorghum porridge consumed predominantly in South Africa and Botswana (Adebo *et al.*, 2018). Due to this phenomenon, the biological basis of fermentation has been largely investigated over the years which has also expanded the focus from initially viewing fermentation primarily as a method of preserving food to a value-adding technique that can improve the nutritional and health properties of food such cereals, fruits, cereals and legumes (Adebo *et al.*, 2018; Ogodo *et al.*, 2019; Rousseau *et al.*, 2020).

Fermented finger millet-based products play a crucial role in the diet of African communities where they contribute as staple and complementary foods for adults and infants, respectively

(Gabaza *et al.*, 2018a). Porridges and beverages such as *ambali* from India, *mahewu* from Southern Africa, *togwa* from Tanzania, and *jnard* from North India are common products made from finger millet. These are still largely prepared following the traditional process, that is, spontaneous fermentation or back slopping (Mugula, Nnko, Narvhus & Sørhaug, 2003; Gabaza *et al.*, 2018a), hence the variations in flavour and microbial properties reported. Although the basic process for the preparation of finger millet-based products starts with basic batter preparation, the proportion of ingredients used, fermentation conditions, and time may vary from place to place (Blandino *et al.*, 2003; Ray, Ghosh, Singh & Mondal, 2016). Details on the traditional preparation process of some common finger millet-based beverages are described briefly below. The associated microbes for each of the fermented products are also given in Table 2.1.

2.6.1 *Ambali*

Ambali, also known as *koozh* (Antony & Chandra, 1997) is a fermented breakfast meal of Central India prepared from finger millet (*E. coracana*) and rice (*Oryza sativa*) composite. It is prepared following a two-step process starting with the preparation of a thick batter from finger millet flour, which is fermented naturally at room temperature for 14 – 16 hours. Following fermentation, partially cooked rice is added to the slurry and cooked. Sour milk is sometimes added to the cooled slurry after which it is ready to serve (Blandino *et al.*, 2003). A three-step process has also been reported where the cooked finger millet and rice slurry is subjected to a second fermentation stage instead of adding sour milk (Antony & Chandra, 1997).

Generally, *ambali* is low in energy, easily digestible, rich in protein, vitamins, and some fatty acids, therefore common for both infants and adults (Blandino *et al.*, 2003; Satish Kumar, Kanmani, Yuvaraj, Paari, Pattukumar & Arul, 2013). Like other cereal-based products, the biochemical modification and bio-enrichment of the product can be attributed to the microbial diversity associated with the product. For example, it is due to the action of microbes that the concentration of carbohydrates and some non-digestible polysaccharides are reduced. Essential amino acids and vitamins are also synthesised during microbial metabolism (Blandino *et al.*, 2003) Some of the microbes associated with *ambali* fermentation include *Leuconostoc mesenteroides*, *L. fermentum*, and *Streptococcus faecalis* (Ray *et al.*, 2016).

2.6.2 *Mahewu*

Mahewu, commonly known as *amahewu* in Zimbabwe, is a non-alcoholic cereal-based beverage that is consumed in Southern Africa. Traditionally, it is prepared from corn/maize meal slurry mixed with finger millet, bulrush millet or sorghum malt and sometimes wheat flour, and left to ferment spontaneously at room temperature (Simango & Rukure, 1991; Mutasa & Ayebo, 1993; Gadaga, Mutukumira, Narvhus & Feresu, 1999) until the desired sourness is achieved. The malt is an important source of inoculum and enzymes such as α-amylase which hydrolyses the pregelatinized starch, thus producing a beverage of acceptable viscosity (Mugocha, 2001). In the rural regions of Zimbabwe, the beverage is common especially in summer or around the planting season where it is served cold in earthen vessels as a refreshment. The predominant microflora associated with the spontaneous fermentation of *mahewu* includes *L. mesenteroides*, *L. brevis*, and *Lactococcus lactis* subsp. *lactis* (Mugocha, 2001; Blandino et al., 2003).

The traditional mahewu making process has been adopted, with some modifications, on an industrial scale for the urban population in Zimbabwe and South Africa (Mutasa & Ayebo, 1993; Gadaga *et al.*, 1999; Mugocha, 2001) where the product is sold in different flavours under different brand names. Powdered *mahewu* concentrates which can prepared for drinking by mixing with water to make an instant beverage or fermented for a shorter period are also available (Mutasa & Ayebo, 1993), although inferior to the traditionally fermented product in terms of sensory attributes (Gadaga *et al.*, 1999). In this case adapted starter cultures are used, instead of the natural uncontrolled process, to eliminate undesirable microflora, improve fermentation time, and produce fermented products of consistent quality (Mugocha, 2001).

2.6.3 *Togwa*

Millet, sorghum, maize, maize-sorghum *togwa* are important fermented food in Tanzania, prepared at household level as weaning food for infants or diluted and served as a refreshment for the adult populace. Just like most African fermented cereal-based products, it is prepared following the spontaneous fermentation process in the rural regions of Tanzania (Mugula *et al.*, 2003). Following the traditional method, *togwa* is prepared by first making a batter from cereal flour (sorghum, maize, millet flour, or a mixture) and water. The mixture is boiled while stirring to avoid lumps then cooled to room temperature. Back slopped *togwa* and cereal

(sorghum or millet) malt are added and mixture fermented for 9 – 24 h (Mugula *et al.*, 2003). The fermented batter is consumed after fermentation without any further processing.

In a laboratory-scale fermentation study by Mugula *et al.* (2003), the *togwa* fermentation process was found to be dominated by LAB and yeast, which suggested a symbiotic relationship between LAB and yeast. The organisms included *L. plantarum*, *L. brevis*, *L. fermentum*, *L. cellobiosus*, *P. pentosaceus*, *W. confusa*, *Issatchenkia orientalis*, *Saccharomyces cerevisiae*, *Candida pelliculosa*, and *C. tropicalis*.

Table 2.1. Microorganisms associated with cereal-based fermentation in selected Asian and African countries

Product	**Country**	**Substrate**	**Microorganisms involved**	**References**
Ambali	India	Rice (*O. sativa*) and Finger millet (*E. coracana*)	*Leuconostoc mesenteroids*, *Lactobacillus fermentum* and *Enterococcus faecalis*	Blandino *et al.* (2003)
Mahewu	Zimbabwe, South Africa	Maize, sorghum, or millet	*L. delbrueku* and *L. bulgaricus*	Karovičová and Kohajdova (2007)
Rabadi	India	Barley, pearl millet, corn or soyabeans, and buttermilk	*Bacillus* sp., *Micrococcuss* sp.	Blandino *et al.* (2003)
Mawe’	West Africa	Maize	*L. fermentum*, *Weissella confusa* and *Pediococcus acidilactici*, *Kluyveromyces marxianus*, *Pichia kudriavzevii* and *Saccharomyces cerevisiae*	Nout (2009); (Houngbédji, Johansen, Padonou, Akissoé, Arneborg, Nielsen, Hounhouigan & Jespersen, 2018)

Ben- saalga	Burkina Faso	Pearl millet (*Pennisetum glaucum*)	*L. plantarum, L. fermentum,*	(Songré-Ouattara, Mouquet-Rivier, Icard-Vernière, Humblot, Diawara & Guyot, 2008)
Koko	West Africa	Pearl millet (*P. glaucum*)	*W. confusa, L. fermentum, L. salivarius* and *Pediococcus* spp.	Lei and Jakobsen (2004)
Enjara	Ethiopia	Sorghum	*Candida guillienmandi*	Karovičová and Kohajdova (2007)
Ting	South Africa	Sorghum	*L. lactis, L. curvatus, W. cibaria, L. fermentum, L. platarum, L. rhamnosus*	Madoroba (2011)
Togwa	Tanzania	Maize, sorghum, finger millet, and maize-sorghum	*L. plantarum, L. brevis, L. fermentum, L. cellobiosus, P. pentosaceus, W. confusa, Issatchenkia orientalis, S. cerevisiae, C. pelliculosa* and *C. tropicalis*	(Mugula *et al.*, 2003)

2.7 Biochemistry of lactic acid fermentation

Lactic acid fermentation is a biochemical process in which LAB transform carbohydrates into organic acids and carbon dioxide. The LAB can either occur endogenously in the raw food or added exogenously as starter cultures (Nout & Motarjemi, 1997). It can be classified into two categories, based on the metabolic by-products, which are homofermentative and heterofermentative lactic acid fermentation. Homofermentative LAB metabolise glucose via the Embden-Meyerhof pathway (glycolysis) to almost exclusively lactic acid (lactate) [Figure 2.5].

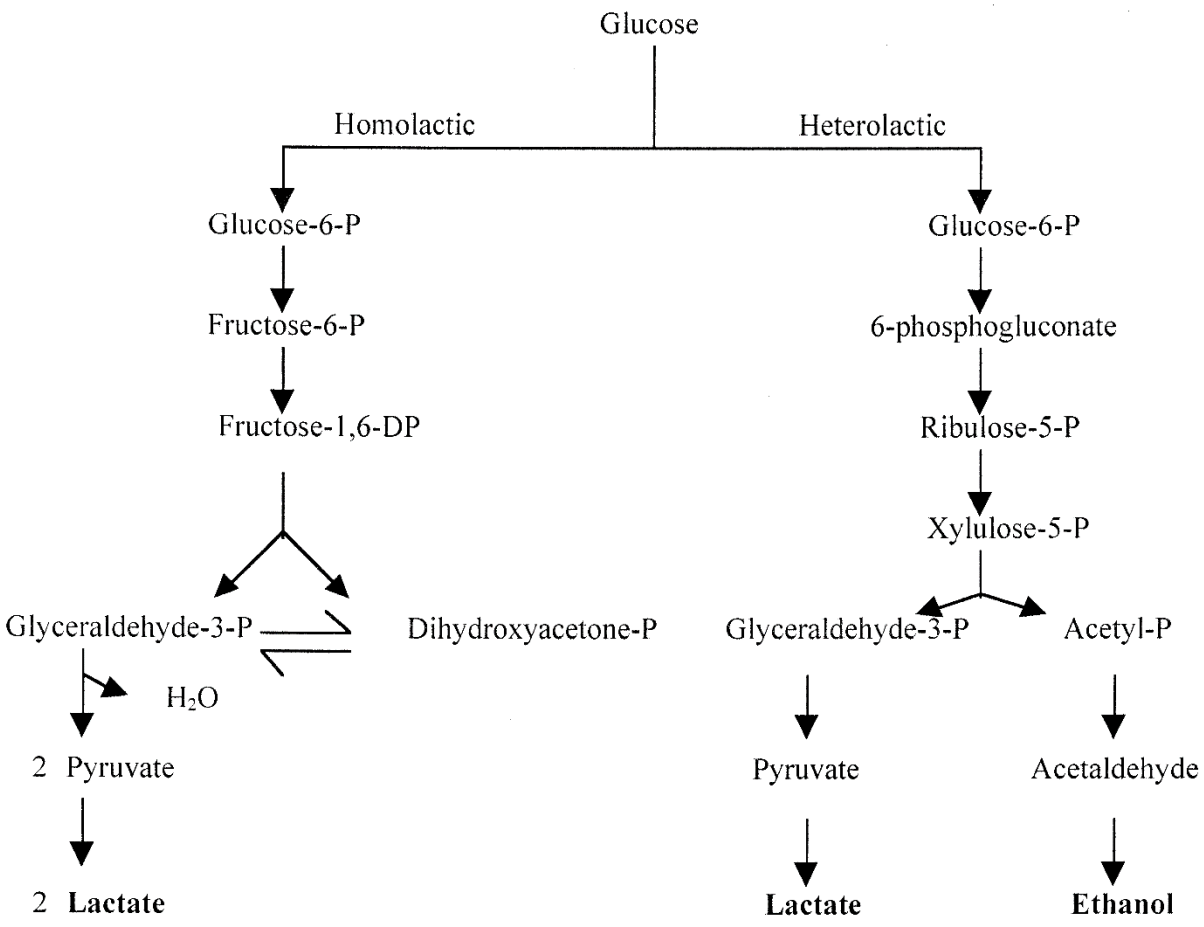


Figure 2.5. Generalised metabolic pathway for homofermentative and heterofermentative lactic acid fermentation. P, phosphate; ADP, adenosine 5'-diphosphate; ATP, adenosine 5'-triphosphate; NAD+, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide (reduced form); (1), lactate dehydrogenase; (2), alcohol dehydrogenase (Caplice & Fitzgerald, 1999)

Homofermentative organisms such as those belonging to the genera *Lactococcus* and *Pediococcus* possess the enzyme aldolase hence the fermentation of glucose directly to lactic acid. The enzyme cleaves fructose -1,6-diphosphate between C_3 and C_4 to produce triose phosphates: dihydroxyacetone phosphate and D-glyceraldehyde-3-phosphate (Figure 2.5). The resultant pyruvate, which is produced from the dephosphorylation of triose phosphate, is decarboxylated to lactate and other by-products such as acetic acid, ethanol, and carbon dioxide at very low or undetected levels depending on oxygen supply (Marshall, 1987; Doelle, 2014). Unlike homofermentation, heterofermentative organisms such as those belonging to the genera *Leuconostoc* use the enzyme phosphoketolase to catabolise glucose to ethanol, carbon dioxide, and lactate in the molar ratio 1:1:1 via the pentose phosphate pathway (Hofvendahl & Hahn–Hägerdal, 2000; Mugocha, 2001; Yun, Wee & Ryu, 2003). Lactate is formed through direct pyruvate reduction by lactate dehydrogenase while ethanol is formed by reduction of acetaldehyde in the presence of alcohol dehydrogenase (Mugocha, 2001).

2.8 Role of starch in lactic acid fermentation

Starch is the major carbohydrate found abundantly in plant sources such as cereals, tubers, and legumes (Xu, Yan & Feng, 2016; BeMiller, 2018). It exists naturally in plants as granules of different shapes and sizes which are concentrated mainly in the endosperm of grains (Singh, Singh, Kaur, Sodhi & Gill, 2003; Svihus, Uhlen & Harstad, 2005). Starch granules are composed of two macromolecular fractions: amylose and amylopectin, made up of glucose polymers which vary in length and degree of branching. Amylose is mostly a linear polymer composed of α-1,4- linked glucose residues while amylopectin is a branched polymer composed of α-1,4- and α-1,6-glycosidic linkages. Within the starch granules, amylose and amylopectin form amorphous and crystalline regions giving them a semi crystalline nature (Tharanathan, Muralikrishna, Salimath & Rao, 1987; Tharanathan, 1995; Oates, 1997). The proportion of amylose to amylopectin, the molecular structure of the amylose and amylopectin fractions, grain size, diameter, shape, and interaction of starch with other non-carbohydrate compounds influences the performance of the cereal grain during processing (Bao, 2019) as well as the concentration of residual material such as glucose, dextrin, and lactic acid during fermentation (Fox, 2018).

Starch is a universal carbon source for the growth of fermenting organisms. It is the main substrate that is degraded by enzymes into fermentable sugars, which are important for organic

acid production (Liu, Ren, Zhao, Jiang, Hao, Jin, Zhang, Guo, Lei & Sun, 2015; Fox, 2018) amongst other by-products. Some of the amylolytic enzymes that have been harvested from microbes and plants, which are capable of hydrolysing starch include α-amylase and glucoamylase. Alpha-amylase is an endo-acting enzyme that hydrolyses at random, the α-1, 4-glycosidic linkages, and some branched α-1, 6-glycosidic linkages of raw starch into branched and unbranched maltodextrins while glucoamylase is an exo-acting enzyme which hydrolyses α-1, 4-glycosidic linkages of starch to glucose (Xu *et al.*, 2016). In some instances, pre-gelatinised starch is used instead of raw starch to improve susceptibility to enzyme digestion and to eliminate microbial competition in starter culture mediated fermentation (Mugocha, 2001). Generally, the first few hours of fermentation can be characterised by an increase in the concentration of simple sugars due to sequential hydrolysis of starch into fermentable sugars by enzymes. In the presence of bacteria and yeast, the simple sugars are readily metabolised into lactic acid, carbon dioxide, ethanol, and other organic compounds which give a characteristic flavour and aroma to fermented products (Evans, Collins, Eglinton & Wilhelmson, 2005; Xu *et al.*, 2016).

Microbial ecology, growth dynamics, and fermentation capacity, associated with starch metabolism, have been investigated and shown to be dependent on the starch source. In a study by Harlow, Donley, Lawrence and Flythe (2015), maize promoted the growth of amylolytic bacteria, predominately *E. faecalis*, 10 and 1000-fold more than wheat and oats, respectively, at 24 h of fermentation. However, a decrease in lactobacilli and lactate-utilizing bacteria was observed for maize compared to the other two substrates. This trend was also similar for wheat except that it had a higher population of lactobacilli compared to maize (Harlow *et al.*, 2015). In terms of fermentation capacity, oat fermentation had a higher gas production rate hence more available as a substrate than maize and wheat. These observations can be linked to differences in the structure and composition of starch granules. Oats starch granules have a higher surface area (about twice as much) than wheat and maize starch granules which allows for better enzyme activity and fermentation capacity (Bowman & Firkins, 1993; Sujka & Jamroz, 2007). Therefore, starch source can determine microbial and physicochemical properties of soured cereal-based products.

2.9 Microbial diversity associated with cereal-based lactic acid fermentation

Starch containing substrates such as cereals are a good medium for the growth of different microbial species which mediate the fermentation process. These microorganisms can be derived naturally from the substrate (spontaneous) or added in the form of starter cultures in the case of controlled fermentation (Simango, 1997; Holzapfel, 2002; Vaidya & Sheth, 2011). Apart from preservation and organoleptic modification of the substrate into a range of products, fermentation organisms play a vital role in natural bio-fortification, bio-enrichment of food as well as health promotion and disease prevention (Steinkraus, 1997; Holzapfel, 2002). This is attributed to production of nutrients like vitamins, amino acids, fatty acids and minerals, degradation of anti-nutrients, and enrichment of functional metabolites such as phenolic compounds, prebiotics, and probiotics by fermentation microflora (Simango, 1997; Steinkraus, 1997). It is on this basis that traditional fermented foods have received considerable attention over the years coupled with intense scientific research and evidence thus increasing consumer awareness and interest in the benefits of fermented foods.

Different microbial species are closely associated with cereal fermentation especially LAB yeasts, and moulds. LAB are Gram-positive and rod-shaped functional group of organisms named after their ability to metabolise carbohydrates to yield lactic acid as the main organic acid. These organisms can proliferate under low pH conditions of up to 5.0 and have the characteristics of being catalase-negative, nonmotile, non-spore-forming, and anaerobic, although they can also grow under microaerophilic conditions (Klein, Pack, Bonaparte & Reuter, 1998; Satish Kumar *et al.*, 2013). *Lactobacillus* and *Bifidobacterium* are amongst the widely researched LAB due to their probiotic properties. They are widely associated with the prevention of diarrhoea, promoting the proliferation of healthy microbiota in the gut while suppressing the growth of pathogenic microbial species and inducing an immune response by activating macrophages (Fuller, 1989; Simango & Rukure, 1992).

In addition to LAB, yeasts of the genera *Saccharomyces*, *Candida*, *Hansenula*, *Saccharomycopsis,* and moulds such as *Aspergillus*, *Rhizopus*, *Mucor*, and *Penicillium* play a significant role in cereal fermentation (Nout, 2009). They are often associated with the production of α-amylase and glucoamylase which aid with the hydrolysis of starch into simple sugars for fermentation. Also, yeasts produce carbon dioxide and organic compounds such as esters, alcohols, glucosides, and acids which contribute to the organoleptic qualities and

improved shelf life properties of fermented products (Ray *et al.*, 2016). Some of the microbial species associated with cereal-based fermentation are summarised in Table 2.1.

2.10 Effects of fermentation on nutritional properties of cereals

Cereals are important sources of dietary nutrients for people all over the world. Their nutritional quality is generally inferior compared to animal products like milk and meat due to their lower protein content, deficiency of some indispensable amino acids, and abundance of antinutritional factors that reduce the availability of nutrients. Fermentation is a simple, effective, and inexpensive biotechnological approach that can be used to substantially improve the nutritional properties of cereals, although nutritional losses have also been reported during fermentation (Blandino *et al.*, 2003). The significant biochemical modification of food during fermentation, which brings forth the desirable nutritional qualities, is due to the combined effect of microorganisms and enzymes produced therein, on the plant tissue (Campbell-Platt, 1994). The effect of fermentation on proteins, minerals, and antinutritional properties is discussed below.

2.10.1 Protein content and quality

Generally, the composition of amino acids, digestibility, and extent of absorption of hydrolysis products (amino acids and small peptides) are basic indices that are used to determine the quality of protein in cereals. While animal proteins are characterised by high levels of essential amino acids, cereal protein are generally of poor-quality due to low levels and ratios of essential amino acids, especially lysine, as well as low digestibility of the cereal protein due to association with antinutritional factors such as phytate and phenolic compounds. Processing techniques that trigger modification of cereal protein structure and improved accessibility by digestive enzymes are important especially for people with marginal or sub-marginal protein intake (Joye, 2019; Duodu, 2019).

Fermentation can improve the protein content and quality of cereals. Significant increases in protein content (10.39-13.97%) and *in vitro* protein digestibility (IVPD) up to 84.28% were reported in LAB fermented sorghum flour with an increase in fermentation time (Ogodo *et al.*, 2019). This improvement can be attributed to a proportional increase in protein concentration due to a proportional decrease in substrate components, hence the high protein content of the fermented food compared to the parental substrate. In a study by Taylor and Taylor (2002), fermentation of sorghum resulted in an increase in IVPD, which coincided with a reduction in

pH and an increase in acidity. Fermentation also improved IVPD of pearl millet from 51% to 80-90%, which was dependent, to a great extent, on the type of bacteria and yeast combination employed. The combination that was shown to result in higher IVPD increase was that of *S. cerevisiae* and *L. fermentum* (Khetarpaul & Chauhan, 1990). General improvement in the lysine and methionine contents of maize flour slurry has been observed during mixed starter culture fermentation which somewhat confirmed the amino acid production potential of *S. cerevisiae* and *L. brevis* in this particular study (Teniola & Odunfa, 2001).

The accumulation of organic acids and the subsequent drop in pH during fermentation are seen to favour the activation of cereal endogenous enzymes such as peptidases and phytases. The hydrolysis of proteins into short-chain peptides and amino acids by peptidases and release of bound proteins through phytase action increases the solubility of proteins and access to digestive enzymes (Chavan, Chavan & Kadam, 1988; Nout & Motarjemi, 1997; Blandino *et al.*, 2003; Annor, Tyl, Marcone, Ragaee & Marti, 2017). Also, a reduction in pH due to acid production may result in structural modification of cereal proteins, which improves their susceptibility to proteolytic enzymes (Taylor & Taylor, 2002). The gradual loss of dry matter, mainly carbohydrates, can also be responsible for the marginal change in protein content (Osman, 2011) especially that which is observed at an advanced stage of the fermentation process when most of the carbohydrates have been catabolised.

Although improvement in protein content is ideal, fermentation has also been found not to have an effect on the protein content of beverages made from sorghum flour (Osman, 2011; Adeyanju *et al.*, 2019). In the production of *ting*, a firm sorghum porridge of South Africa and Botswana, the protein content of porridges prepared from five different sorghum varieties remained more or less constant throughout the fermentation process. This suggested the conservation of protein while the preferred substrate, the carbohydrate component, was presumably converted to organic acids by microflora (Taylor & Taylor, 2002). Undesirable losses in protein content or amino acids during fermentation have however been reported (El Hag, El Tinay & Yousif, 2002; Osman, 2011; Assohoun, Djeni, Koussémon-Camara & Brou, 2013). This has been attributed to microbial hydrolysis of amino acids to volatile compounds such as ammonia (Pranoto, Anggrahini & Efendi, 2013), which is unfavourable when protein malnutrition is a matter of concern.

2.10.2 Mineral bioaccessibility and mineral inhibitors

Fermentation has been widely advocated as an effective processing approach that can be used to improve the availability of minerals from staple cereal-based foods. Although evidence exists to support the phenomenon, inconsistent results have been reported on the effect of fermentation on mineral bioaccessibility. For example in a fermentation study by Gabaza *et al.* (2018a), no improvements were observed in iron and zinc bioaccessibility of finger millet porridges although mineral inhibitors (phenolic compounds, condensed tannins, and phytate) were reduced by up to 41%, 35%, and 22-54%, respectively, during the process. This shows that the reduction in mineral inhibitors does not automatically translate to improved mineral bioaccessibility. Also, the fermentation of rice and black gram batter for *idli* and *dosa* preparation did not have an effect on the bioaccessibility of copper. The shorter fermentation time (12 h) used in their study seemingly did not favour sufficient degradation of phytic acid to cause significant improvements in copper bioaccessibility (Kumari & Platel, 2020).

According to Hurrell and Egli (2010), at least 90% phytate degradation is required to cause a two-fold increase in bioavailable iron. More so the phytate/mineral molar ratio must be very low (< 0.4 for cereal-based foods with no iron enhancers) if at all mineral bioaccessibility can be improved (Hurrell, 2004; Hurrell & Egli, 2010). However, in some whole grain high-tannin sorghum and millet gruels, a lower phytate/mineral molar ratio may not result in an increase in mineral bioaccessibility due to the presence of other inhibitors like tannins and dietary fibre. These inhibitors can interact with previously released minerals which further inhibits mineral bioavailability (Sripriya, Antony & Chandra, 1997; Osman, 2011; Gabaza *et al.*, 2017). This may explain why in some cases degradation of phytate is not always followed by an increase *in vitro* mineral bioaccessibility (Mohite, Chaudhari, Ingale & Mahajan, 2013).

On the contrary, Adeyanju *et al.* (2019) reported higher bioaccessible iron (8-192%) and zinc (24-28%) in fermented sorghum beverages compared to unfermented beverages which corresponded with a reduction in phytate content of up to 83%. In this case, the acidic environment created during fermentation presumably created a favourable condition for endogenous phytase activity. The hydrolysis of phytate therein to lower inositol phosphates may have released bound divalent ions making them more bioaccessible. Also, organic acids produced as metabolic by-products can enhance the bioaccessibility of minerals (iron and zinc) through formation of soluble complexes (Tontisirin, Nantel & Bhattacharjee, 2002; Gabaza *et*

al., 2018a). Similarly, Sripriya *et al.* (1997) reported an increase in bioavailable calcium, phosphorus, and iron in fermented finger millet which was likely due to the degradation of mineral inhibitors.

2.11 Concluding remarks and gaps in knowledge

Cereal grains are important sources of dietary nutrients and have the potential to improve the nutritional and health status of people in SSA, especially when used as whole grains. Despite their relatively good nutritional profile, they are sometimes inferior when compared to animal products due to deficiency of essential amino acids like lysine and abundance of antinutritional factors such as phytate, phenolic compounds, especially tannins, which bind to mineral and protein, adversely affecting their bioavailability in the human body. More so, the shift from traditional whole grains to refined cereal-based diets is contributing to the sharp rise in diet-related NCDs.

Fermentation is a cost-effective approach that can be used to improve the nutritional and health properties of cereal-based products. This may help to reduce the burden of macronutrient, micronutrient deficiencies, and NCDs experienced in SSA. Finger millet is an important cereal crop in the arid and semi-arid regions of Asia and Africa and is highly valued for its nutritional properties and drought tolerance. While much is known about the fermentation benefits of finger millet, little is known about how adding extra sources of starch during fermentation influences the microbiological, nutritional, and bioactive properties of finger millet-based beverages.

Starch is the main substrate that is degraded by enzymes to fermentable sugars whose availability for microbial hydrolysis during fermentation may improve fermentation rate and accumulation of organic acids, amongst other by-products. The subsequent drop in pH thereof may trigger a favourable modification of the food matrix microstructure which may lead to even better solubility of nutrients and bioactive compounds, hence more available for metabolism in the human body. Starch slurries extracted from cereals such as rice and maize during cooking need to be investigated for their potential to improve microbial diversity and fermentation rate when composited with other indigenous cereal crops such as finger millet, which may subsequently enhance the nutritional and health properties of fermented cereal-based products.

2.12 Hypotheses and Objectives

2.12.1 Hypotheses

1. Soured finger millet-based slurries produced by spontaneous fermentation with the inclusion of rice starch will have a higher fermentation rate and microbiological diversity compared to the formulation that uses maize starch. Due to a smaller starch granule size, which increases the surface area for digestion, rice starch (Ali, Wani, Wani & Masoodi, 2016) will have a greater extent and rate of *in vitro* starch digestibility by fermentation organisms, increasing fermentation rate and microflora diversity. The opposite will be true for finger millet-based slurries to which the larger granule size maize starch (Svihus *et al.*, 2005; Ren, Chen, Molla, Wang, Diao & Shen, 2016) will be added under the same conditions.

2. Soured finger millet-based beverages with added rice starch will have higher protein digestibility and mineral bioaccessibility than soured finger millet-based beverages with added maize starch. Phytic acid degradation in finger millet will be better in rice fermentation due to higher fermentation rate and activity of fermentation microflora. This will provide optimum pH conditions for enzymic degradation of phytic acid present in the form of complexes with minerals and proteins. Such a reduction in phytate content will increase the amount of soluble minerals and digestibility of proteins in the human gut (Blandino *et al.*, 2003; Towo, Matuschek & Svanberg, 2006).

3. Soured finger millet-based beverages with the inclusion of rice slurry will have a higher total phenolic content and antioxidant activity compared to the formulation that uses maize starch. Due to better fermentation rate and simultaneous increase in acidity of the rice formulation, an increase in the concentration of free phenolic compounds in finger millet is expected owing to the reorganization and abstraction of hydride ions in phenolics at low pH conditions. (Motarjemi & Nout, 1996; Gabaza *et al.*, 2016). An increase in the concentration of free phenolic compounds in the rice formulation will have the resultant effect of enhanced antioxidant properties against free radicals.

2.12.2 Objectives

1. To determine the influence of starch source used during spontaneous fermentation on microbiological diversity of finger millet-based slurries with the aim of gaining an

understanding of the microbiological ecology of fermented finger millet-based slurries with added rice and maize starch.

2. To determine the effect of using different sources of starch on protein quality and mineral bioaccessibility of soured finger millet-based beverages with the aim of producing a soured finger millet-based beverage with enhanced nutritional qualities.

3. To determine the effect of varying the starch source during fermentation on phenolic compounds and antioxidant properties of soured finger millet-based beverages with the aim of producing functional foods with enhanced health-promoting properties.

CHAPTER 3: RESEARCH

3.1 Microbiological diversity of finger millet-based slurries as influenced by starch source during lactic acid fermentation

Abstract

In this research, the effect of including exogenous starch sources on microbial profile and diversity during spontaneous fermentation of finger millet-based slurries was investigated. The fermented samples studied were finger millet only control (C), finger millet with added maize starch (M), and finger millet with added rice starch (R) slurry at 0, 7, and 14 h of fermentation. The resultant beverages were analysed for biochemical properties and enumerated for microbial counts. Preliminary identification of lactic acid bacteria (LAB) isolates and genotypic characterisation after incubation at 28°C was done by Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) fingerprinting and 16S ribosomal ribonucleic acid (16S rRNA) amplicon sequencing, respectively. The fermentation process was dominated by yeast and LAB, along with a decrease in pH and an increase in titratable acidity, particularly in the last phase of the process. At the onset, Enterococcal and Lactobacillus species dominated particularly *Enterococcus casseliflavus*, *E. faecium*, *Lactobacillus plantarum*, *L. amylophilus*, and *L. paracasei* ssp. *paracasei* some of which were also abundant at the end of fermentation. The predominant genera for all the formulations at 14 h of fermentation were of the order *Lactobacillales*. Specifically, *Weissella confusa* (Leuconostocaceae family) increased from 0.225% at 0 h to 79.80% after 14 h of fermentation and followed by the genera *Enterococcus* from 5.25% to 22.28%. There were no differences in bacterial population, species diversity or abundance (alpha diversity), and beta diversity indexes, among the fermentation groups. Overall, the starch source did not affect the microbial profile and diversity of fermented finger millet-based slurries. The study provided an in-depth knowledge of the microbiota variations during natural fermentation of finger millet. This knowledge can be effectively used in the identification of biomarkers (key fermenting bacteria) to assess the quality of fermented foods and to design starter cultures for enhanced nutritional benefits in indigenous cereal crops of Sub-Saharan Africa (SSA).

Keywords: finger millet-based slurries, maize starch slurry, rice starch slurry, lactic acid fermentation, microbial diversity, *Weissella*, *Enterococcus*.

3.1.1 Introduction

Fermented products, including beverages and porridges, continue to contribute significantly to human diets in SSA. They constitute a larger portion of meal intake, especially in middle and low-income groups (McFeeters, 1988; Gabaza *et al.*, 2018a). Of interest are fermented gruels prepared from major indigenous cereals of SSA such as maize and finger millet, which are as staple and complimentary foods (Gabaza *et al.*, 2018a). Although the benefits of optimized starter culture inoculated fermentations have been confirmed, preparation procedures for most African fermented beverages and porridges are still artisanal and by uncontrolled inoculation (Mugocha, 2001). Therefore, spontaneously fermented products are still a significant component of the human diet with the potential to improve health and nutritional status in SSA.

The fermentation of cereals to produce beverages is mediated by a consortium of bacteria and yeasts, which are naturally found in the grains or added as starter cultures (Nout & Motarjemi, 1997). Without specific microbial composition, major differences in the quality and stability of the final beverages can be observed. This has fuelled the interest in the application of biotechnological techniques to develop shelf-stable and standardised fermented products (Mugocha, 2001; Marsh, Hill, Ross & Cotter, 2014). The microbial composition of naturally fermented cereal-based products varies but is predominantly associated with LAB, including *Lactobacillus*, and *Bifidobacterium* (Karovičová & Kohajdova, 2007). In some beverages, yeasts such as *Candida* and *Saccharomyces* have also been detected (Marsh *et al.*, 2014). These organisms provide a natural way of improving the nutritional properties of fermented food while reducing the concentration of undesirable compounds such as antinutrients (Holzapfel, 1997; Holzapfel, 2002). There is also a strong link between microbial composition and population, and health outcomes such as improved gastrointestinal health. While the mechanisms involved may be unclear, the functional benefits of fermented beverages can be directly linked to the probiotic action of microflora and indirectly to the production of metabolic by-products like bioactive peptides and short-chain fatty acids (Marsh *et al.*, 2014; Liu *et al.*, 2015).

The growth of fermenting microflora and the concentration of metabolic by-products can be altered by the composition of the fermentation environment. Hence, it is important to optimize nutrient concentration, especially fermentable carbohydrates to ensure growth of diverse microflora (Gupta, Cox & Abu-Ghannam, 2010). Cereal grains are recognised as high-energy

crops since they rich in carbohydrates such as starch and simple sugars (Harlow *et al.*, 2015). Starch is a highly fermentable substrate and is an important carbon source for the activity and growth of bacteria such as bifidobacteria (Liu *et al.*, 2015) and LAB. In a study by Gupta *et al.* (2010), the concentration of sugar, as a carbon source, was seen to alter the proliferation of *L. plantarum* to a considerable extent. Different botanical sources of starch such as oats, corn, and wheat have also been demonstrated to alter the activity of bacteria such as *Lactobacillus* spp. during fermentation. This can be linked to the physical characteristics of starch granules, their composition (presence of non-starch components), and the presence of enzyme inhibitors (Harlow *et al.*, 2015). Therefore, the abundance of bacteria and metabolites in fermented products can be associated with the concentration of fermentable carbohydrates (Liu *et al.*, 2015) or the microbial fermentative capacity which is dependent on the starch source (Harlow *et al.*, 2015).

The addition of an extra carbohydrate source is a common practice during the preparation of cereal-based beverages. Examples of such beverages include a*mahewu* which are prepared from finger millet or sorghum flour with the addition of maize (Karovičová & Kohajdova, 2007), and *ambali* prepared from finger millet slurry and partially cooked rice to form a batter (Ray *et al.*, 2016). Most of the research to date has explored the fermentation benefits of finger millet (Antony & Chandra, 1998; Makokha *et al.*, 2002; Gabaza, Shumoy, Muchuweti, Vandamme & Raes, 2018b); however, the effect of adding extra starch source during fermentation of finger millet-based beverages is less established. Knowledge of the microbial variations of finger millet beverages with added starch can inform the development of fermented products with enhanced nutritional and functional properties.

Therefore, the present study investigated the effect of adding maize and rice starch on the microbial diversity of fermented finger millet slurries with the hypothesis that the addition of different botanical sources of starch will influence the microbial population of fermented products.

3.1.2 Materials and methods

3.1.2.1 Materials

3.1.2.1.1 Collection and preparation of raw materials

Finger millet grains were obtained from the rural areas of Gwanda in Zimbabwe. Commercially available basmati long-grain white rice (Spekko Indian Gate, Pioneer Foods, Tygervalley, South Africa) and maize grits, also known as "samp" (Ace Samp, Tiger Consumer Brands Limited, Bryanston, South Africa) were purchased from a local supermarket in Hatfield, South Africa. Clean finger millet whole grains were ground to flour using a Laboratory Hammer Mill (Perten, Sweden) fitted with a 500 µm sieve.

3.1.2.1.2 Preparation of rice and maize starch slurries

Starch slurries were prepared by boiling rice and maize samp at a solids to-water ratio of 1:6 and 1:5, respectively until cooked. The excess slurry was drained, collected, and the cooked grains discarded. The extraction of starch slurries from both cereals was consistent and standardised independently following cooking instructions from the manufacturer.

3.1.2.1.3 Preparation of finger millet-based slurries through the spontaneous fermentation process

This was done by mixing whole grain finger millet meal with cooled (room temperature) starch slurry to a thick batter at a ratio of 1:4 (w/v) as illustrated in Figure. 3. 1. The batter was fermented overnight at 28ºC in a thermostat-controlled water bath to achieve a pH of 4.8 to 5.3. Cereal slurry aliquots were pooled at 0 h, 7 h, or 14 h to monitor biochemical and microbiological changes. Finger millet slurries without an additional starch source were used as the control of the experiment. The sample codes and descriptions are tabulated in Table 3.1.

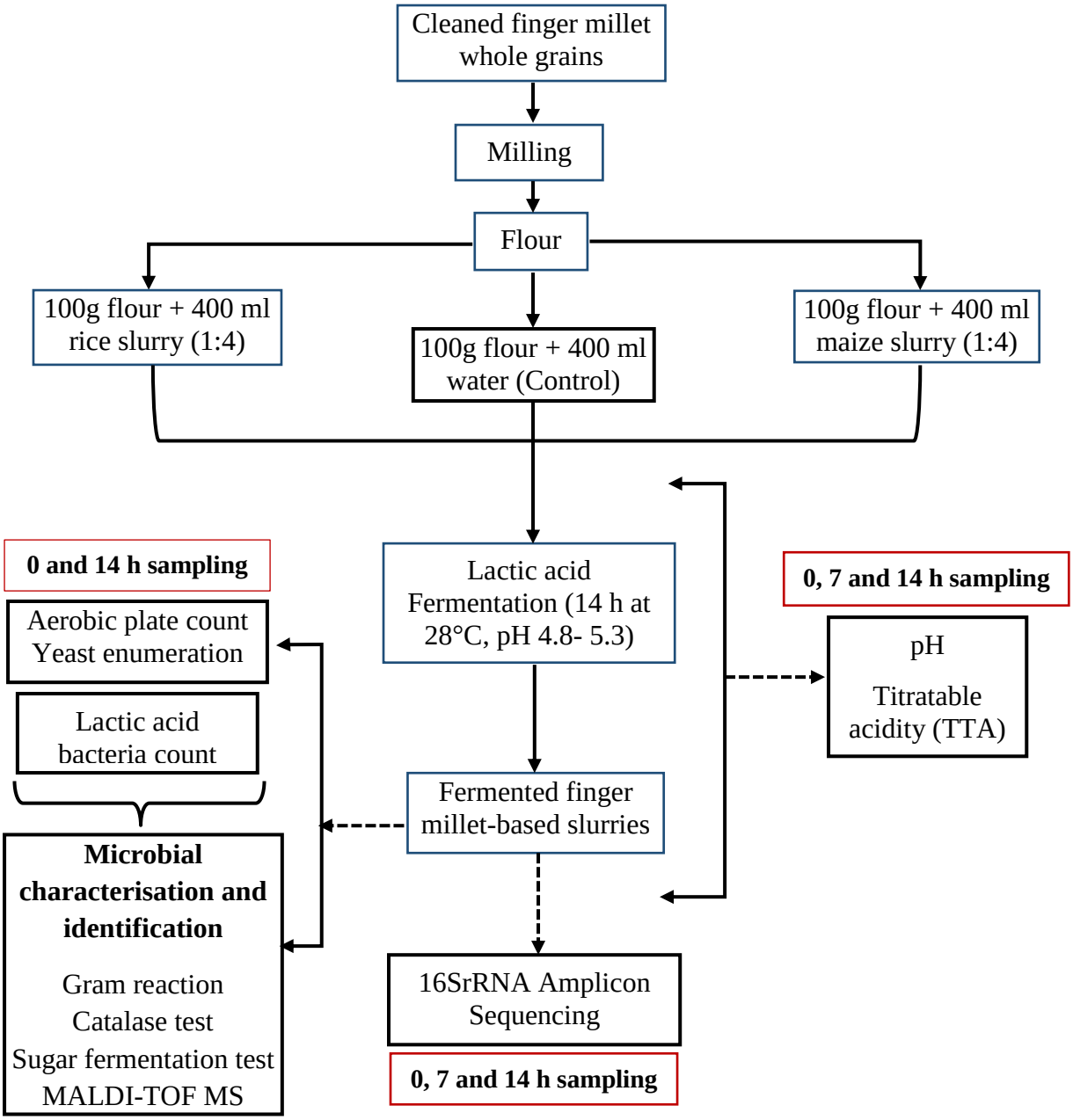


Figure 3.1. Process flow for preparation and characterisation of three fermented finger millet-based slurries: finger millet and maize (M), finger millet and rice (R) and finger millet only (C). Sample aliquots for analysis were drawn at different time intervals depending on analytical technique (0 h, 7 h or 14 h)

Table 3.1. Particulars of sample codes in the fermentation experiment

Sample Formulation	Fermentation Time	Sample ID
Finger millet (C)	0	C0
	7	C7
	14	C14
Finger millet and rice starch (R)	0	R0
	7	R7
	14	R14
Finger millet and maize starch (M)	0	M0
	7	M7
	14	M14

Note: The sample ID assigned here will be used in the entire chapter for describing the methodology, results, and discussion.

3.1.2.2 Analytical methods

3.1.2.2.1 pH and titratable acidity

Measurement of pH was done using an Instrulab digital pH meter (Hanna Instruments pH 211 Microprocessor). Titratable acidity (TTA), expressed as % lactic acid/ g of sample, was measured from an aliquot (5 ml) of fermented cereal slurry solution against 0.1 M NaOH, with phenolphthalein solution as an indicator (Antony & Chandra, 1997).

3.1.2.2.2 Microbial enumeration and isolation

Aerobic microorganisms in the finger millet slurries were enumerated at 30°C for 72 h, LAB at 30°C for 72 h and yeast cells at 25°C for 72 h, on Plate Count Agar (CM0325Oxoid, Hampshire, United Kingdom), de Man, Rogosa and Sharp [MRS] Agar (De Man, Rogosa & Sharpe, 1960) and Yeast Extract Agar (LAB018 Acumedia Lab Neogen Culture Media, Lancashire, United Kingdom), respectively. Briefly, 10 ml aliquots from each of the treatments were suspended in 90 ml of 0.1% (w/v) sterile buffered peptone water (HG00C134.500 Biolab Merck, Modderfontein, South Africa) and appropriate 10-fold dilutions plated and incubated. Plates with 30-300 colony forming units (cfu) were counted and results were expressed as Log_{10} (cfu/ml). Colonies on suitable MRS plates and plate count agar were picked and purified by repeatedly streaking on MRS and nutrient agar, respectively, for further screening.

Presumptive LAB cultures less than 24 h old were used for all further biochemical tests (Amoa-Awua & Jakobsen, 1995; Houngbédji *et al.*, 2018).

3.1.2.2.3 Biochemical characterisation of isolates

3.1.2.2.3.1 Gram reaction

A loop full of the colony material was dissolved in a drop of 3% potassium hydroxide (KOH) on a microscope slide while observing for the formation of ropy strings (Houngbédji *et al.*, 2018). The occurrence of ropy strings was associated with Gram-negative organisms, and vice-versa was true for Gram-positive isolates.

3.1.2.2.3.2 Catalase test

Catalase reaction is a test that is used to differentiate bacteria based on the ability to produce the enzyme catalase, which neutralises the oxidative effect of hydrogen peroxide (H_2O_2). Catalase-positive organisms, therefore, can break down H_2O_2 into water and oxygen hence the rapid formation of gas bubbles when exposed to H_2O_2 (Reiner, 2010). To perform the test, a drop of 30% H_2O_2 solution was added onto a fresh mass (< 24 h old cultures) of colony material on a glass slide while observing for the generation of effervescence (Reiner, 2010; Houngbédji *et al.*, 2018).

3.1.2.3.3 Sugar fermentation test

The test was done following a modified procedure by Guessas and Kihal (2004). MRS broth medium and glucose solution to a final concentration of 1% was used. Phenol red was added to give a sufficiently red colour and the basal fermentation media sterilised with inverted Durham tubes submerged in the broth. Each tube was inoculated with fresh culture and gas and/or acid production after incubation at 37°C for 18-24 h was interpreted as evidence for sugar fermentation.

3.1.2.3 Statistical analysis

Microbial enumerations, pH, and titratable acidity data were statistically analysed for significant differences between the treatments, as a function of time, by Multi-factor Analysis of Variance (ANOVA) using Stat graphics Centurion XV. Discrimination of means by multiple comparison procedure was done using Fischer's least significant difference [LSD] test ($P < 0.05$).

3.1.2.4 Rapid Identification of bacterial isolates by Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) Bio-typing

Qualitative analysis of bacterial isolates by MALDI-TOF was done following the direct colon transfer technique as described by Moodley (2015). Only isolates that were Gram-positive, catalase-negative, and capable of fermenting glucose to carbon dioxide were further identified at species level. A total of 90 purified and fresh colonies were individually smeared onto thoroughly cleaned MALDI stainless steel target plates and matrix solution (1 µl), formulated from α-cyano-4-hydrocinnamic- acid [CHCA] (Sigma-Aldrich), applied onto the smear and left to air dry at room temperature. The dry biological formulations were analysed by MALDI-TOF MS (Bruker Daltonik GmbH MALDI Biotyper, Germany) for the unique bacterial cell proteomes and compared to reference microbial spectra already present in the Bruker database for identification. Scores on a scale from 0 to 3.0 exhibited the level of probability identification. Best genus and species probable matches were selected based on confidence score values.

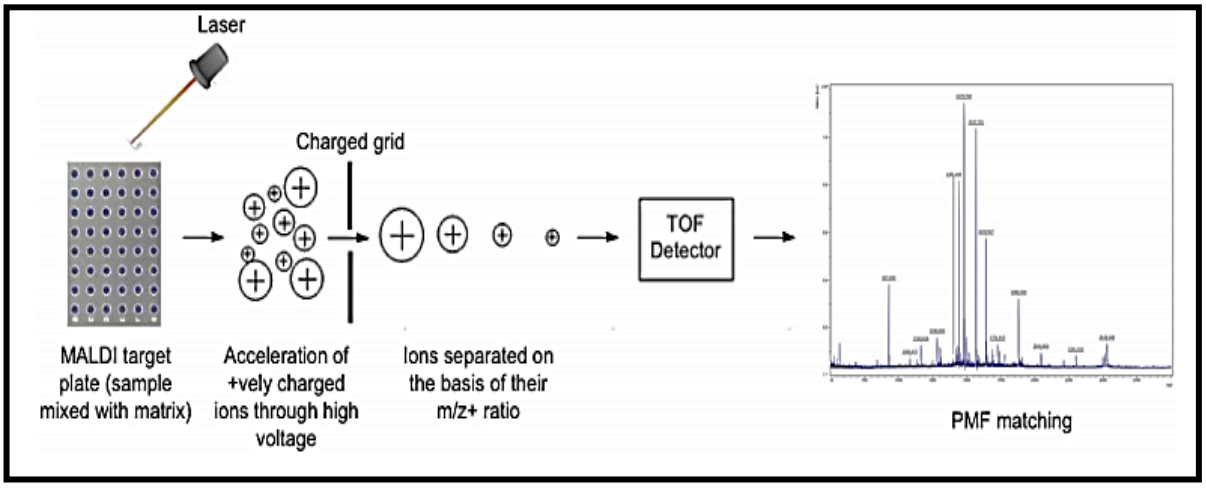


Figure 3.2. Schematic representation of the MALDI-TOF workflow and principle (Singhal, Kumar, Kanaujia & Virdi, 2015). Mass-to-charge ration (m/z+), time of flight (TOF), peptide mass fingerprint (PMF)

3.1.2.5 16s rRNA amplicon sequencing for diversity and succession analysis of microbiota during fermentation

3.1.2.5.1 DNA extraction

DNA extraction from samples at different fermentation time intervals (0, 7, and 14 h) was done using the ZymoBIOMICS™ DNA Miniprep Kit and following the prescribed protocol available from www.zymoresearch.com. To start with, 250 µl of slurries were mixed with 750 µl of ZymoBIOMICS™ Lysis Solution by vortexing at high speed for 5 mins. The sample solids were removed by centrifuging at 10 000 × g for a minute at 4 °C after which 400 µl of the supernatant was filtered by further centrifugation at 8 000 × g for a minute in a filter fitted collection tube. To the filtrate, 1200 µl of the DNA binding buffer solution was added and thoroughly mixed. Duplicate 800 µl aliquots of the mixture were centrifuged at 10 000 × g for 1 min in a collection tube, one at a time, and the flow-through from both aliquots was discarded. The pellet was washed twice with 700 µl and 200 µl of the DNA wash buffer, separately, and the flow-through after centrifuging at 10 000 × g was discarded. The extracted DNA was suspended in 100 µl DNase Free water for 1 minute which was eluded by centrifugation a 10 000 × g for 1 min. The eluded DNA was filtered into a clean microcentrifuge tube at exactly 16 000 × g for 3 min and stored at -20 °C for molecular analysis.

3.1.2.5.2 16S rRNA gene amplicon sequencing and sequence analysis

PCR amplification and sequencing of the 16S rRNA gene V4 variable region was done at MR DNA, Shallowater, TX, USA (www.mrdnalab.com) using the reverse and forward primers 515 and 806, respectively (Caporasoa, Lauberb, Waltersc, Berg-Lyonsb, Lozuponea, Turnbaughd, Fierer & Knight, 2011). The reactions were done using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) at 94 °C for 3 min followed by 30 elongation cycles. Successfully PCR amplicons were pooled together based on molecular weight and DNA concentration and purified using calibrated Ampure XP beads. An Illumina DNA library was prepared from the purified product and sequencing on the MiSeq platform (2 × 250bp) was run with the amplicons. The analysing of the sequenced products for all the finger millet-based samples (18 samples) was done using Quantitative Insights into Microbial Ecology 2 version 2019. 7 Package (QIIME 2 2019.7) (Bolyen, Rideout, Dillon, Bokulich, Abnet, Al-Ghalith, Alexander, Alm, Arumugam & Asnicar, 2018). The imported raw data files were demultiplexed using the q2-demux plugin and denoised using the Divisive Amplicon Denoising Algorithm 2 [DADA2] pipeline (Callahan, McMurdie, Rosen, Han, Johnson & Holmes, 2016) available in QIIME2

2019.7 and assignment of Operational Taxonomic Units (OTUs), within a sequence similarity threshold of 97%, was performed using BLASTn against an NCBI (www.ncbi.nlm.nih.gov) derived database. Estimation of alpha diversity, group evenness, and species richness were done using Shannon's diversity, Pielou's evenness, and Faith's phylogenetic diversity indexes (Faith, 1992). To explain the grouping patterns of samples based on the raw material used and bacterial community diversity, beta diversity Unifrac distance plots were used particularly, Jaccard Unifrac index, Bray Curtis Unifrac matrix, weighted (Lozupone, Hamady, Kelley & Knight, 2007), and unweighted Unifrac matrix (Lozupone & Knight, 2005).

3.1.2.6 Statistical analysis of sequencing data

Alpha diversity comparison between fermentation groups was calculated using the Kruskal-Wallis test script in QIIME 2 2019.7 (Bolyen *et al.*, 2019). Significant differences in beta diversity were analysed using the Permutational Multivariate Analysis of Variance [PERMANOVA] test (Anderson, 2014). Analysis of Composition of Microbiome [ANCOM] test (Mandal, Van Treuren, White, Eggesbø, Knight & Peddada, 2015) was run to detect differences in the percentage relative abundance of taxa between the fermented samples at the different stages of the fermentation process. Microsoft Excel software was used to calculate the mean sequence amplicons and standard deviation per sample. Statistical significance for all analysis was tested at a 95% confidence interval ($p<0.05$).

3.1.3 Results and Discussion

3.1.3.1 pH and titratable acidity during spontaneous fermentation of finger millet-based slurries

Table 3.2. Analysis of variance for effect of starch source and fermentation time on pH and TTA of finger millet-based slurries

	pH		TTA	
Source	Df	P-value	Df	P-value
MAIN EFFECTS				
A: Starch source	2	0.7172	2	0.8251
B: Fermentation time (hours)	2	0.0000	2	0.0000
INTERACTIONS				
AB	4	0.4536	4	0.6625

Table 3.2. shows the two main effects, starch source (A) and fermentation time (B) and their interaction (AB). The starch source as an independent variable did not affect the pH and TTA of finger millet-based samples, however, fermentation time had a statistically very highly significant effect ($p < 0.0000$) on the pH and TTA of samples. The non-significant interaction between starch source and time (AB) shows that the variability in pH and TTA of samples at different fermentation time intervals was independent of the starch source.

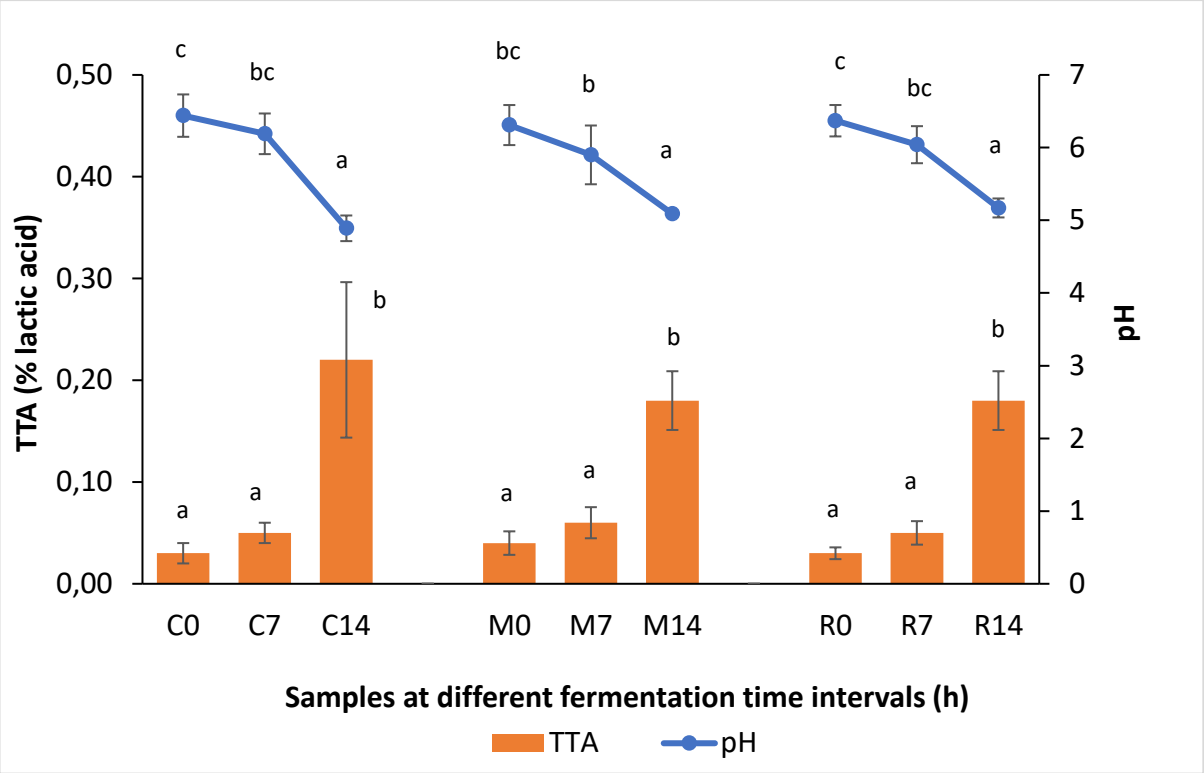


Figure 3.3. Effect of starch source and fermentation time on pH and titratable acidity (TTA) of finger millet-based slurries (n=9). Values are the mean (± SD) of 3×3 replications. Different superscripts for each parameter (pH and TTA) indicate significant differences. The samples are finger millet only (C), finger millet and maize starch (M) and finger millet and rice starch (R) slurry

The effect of starch source and fermentation time on pH and TTA is shown in Figure. 3.3. The pH of finger millet only slurries decreased from 6.44 ± 0.29 to 4.89 ± 0.18. With the addition of rice starch, the pH decreased from 6.37 ± 0.22 to 5.17 ± 0.13, while with the addition of maize starch the pH decreased from 6.31 ± 0.28 to 5.09 ± 0.03. A two-phase trend was observed with the physicochemical changes in all the treatments during the fermentation process. In the first phase (0-7h), there was no difference in pH and acidity of samples with an increase in fermentation time while in the second phase of fermentation (7-14 h), a significant ($p < 0.05$) decrease in pH and an increase in acidity were observed with an increase in fermentation time. However, concerning the objective of this study, the impact of the starch source (rice and maize starch) was not observed in the spontaneous fermentation process. The trend observed, that is, the decrease in pH and increase in TTA with an increase in fermentation time for all fermentation groups has been reported during effective lactic acid fermentation of cereal-based products (Ramos, de Almeida, Freire & Schwan, 2011; Houngbédji *et al.*, 2018; Adeyanju *et*

al., 2019). This occurs mainly due to the acidic environment created by LAB and sometimes yeasts during fermentation (Moodley, 2015).

3.1.3.2 Microbial enumeration during spontaneous fermentation of finger millet-based beverages

Table 3.3. Analysis of variance for effect of starch source and fermentation time on microbial counts of finger millet-based slurries

Source	**Aerobic plate count [APC]**		**LAB**		**Yeast count**	
	Df	P-value	Df	P-value	Df	P-value
MAIN EFFECTS						
A: Starch source	2	0.9301	2	0.0967	2	0.8775
B: Fermentation time (hours)						
INTERACTIONS	1	0.0000	1	0.0000	1	0.0000
AB						
	2	0.0347	2	0.2355	2	0.0310

Table 3.3. shows the two main effects, finger millet-based slurries (A) and fermentation time (B) and their interaction (AB). The starch source as an independent variable (A), did not affect microbial counts, however, fermentation time (B) had a statistically significant effect ($p < 0.05$) on microbial counts. The interaction between starch source and fermentation time (AB) on microbial counts is not significant.

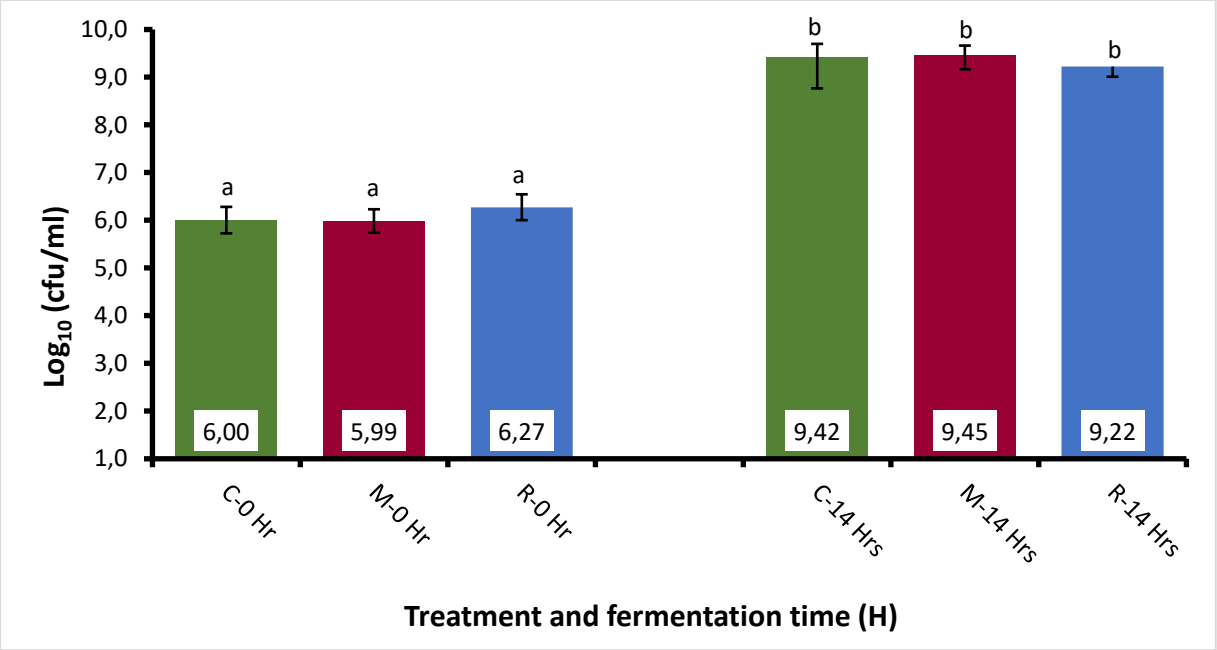


Figure 3.4. Effect of starch source and fermentation time on aerobic plate count of finger millet-based slurries. Letter C represents finger millet only slurry (control), M represents finger millet and maize starch slurry, and R represents finger millet and rice starch slurry. Values are the mean (± SD) of 3×2 replications (n=6). Different superscripts in the same time category indicate significant differences

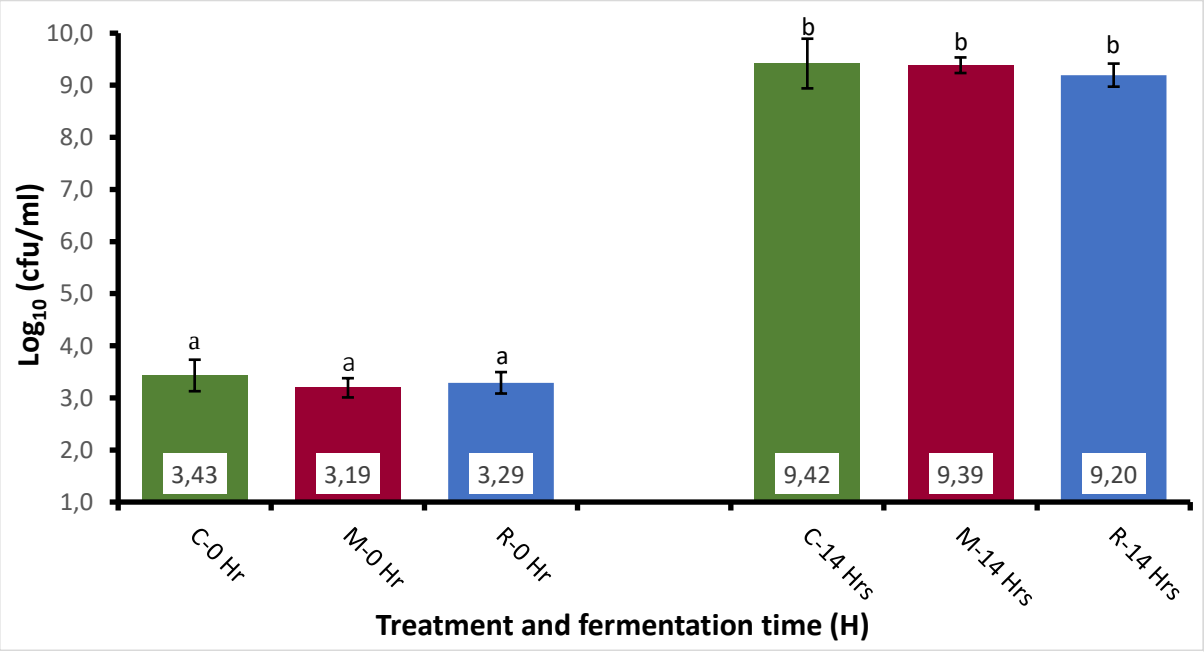


Figure 3.5. Effect of starch source and fermentation time on lactic acid bacteria count of finger millet-based slurries. Letter C represents finger millet only slurry (control), M represents finger millet and maize starch slurry, and R represents finger millet and rice starch slurry. Values are the mean (± SD) of 3×2 replications (n=6). Different superscripts in the same time category indicate significant differences

A general increase in the level of microbes in the fermented products, with or without added starch, as indicated by APC (Figure 3.4) and LAB (Figure 3.5) was observed. The trend in bacterial growth throughout the fermentation process explains and supports the two-phased biochemical changes observed (Figure 3.3). Overall, there were no differences in all microbial counts between treatments before (0 h) and after the fermentation period of 14 h as per the traditional beverage preparation process. Therefore, the conclusion that starch source as an did not alter the bacterial population of fermentation groups. As expected, the initial count of lactic acid bacteria (3 Log cfu/ml) tripled at the end of fermentation (9 Log cfu/ ml) as shown in Figure.3.5. Ogodo *et al.* (2019) reported a consistent increase in total viable count and LAB with time during fermentation of maize and sorghum flour. This was interpreted as evidence of the adaptation of microflora to the substrate used.

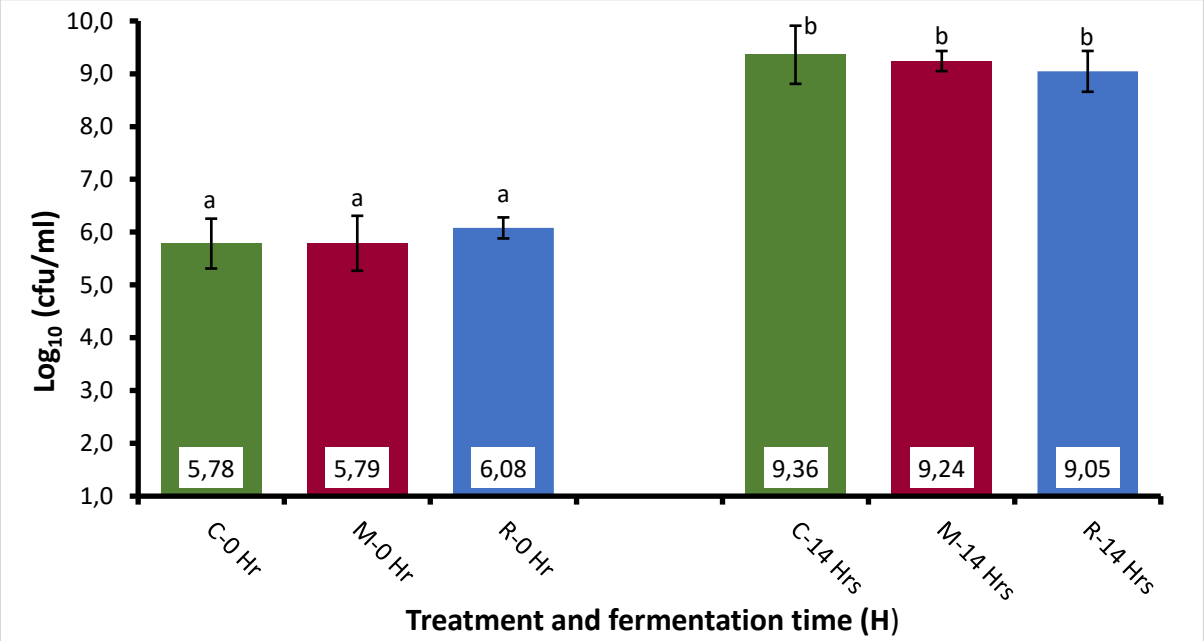


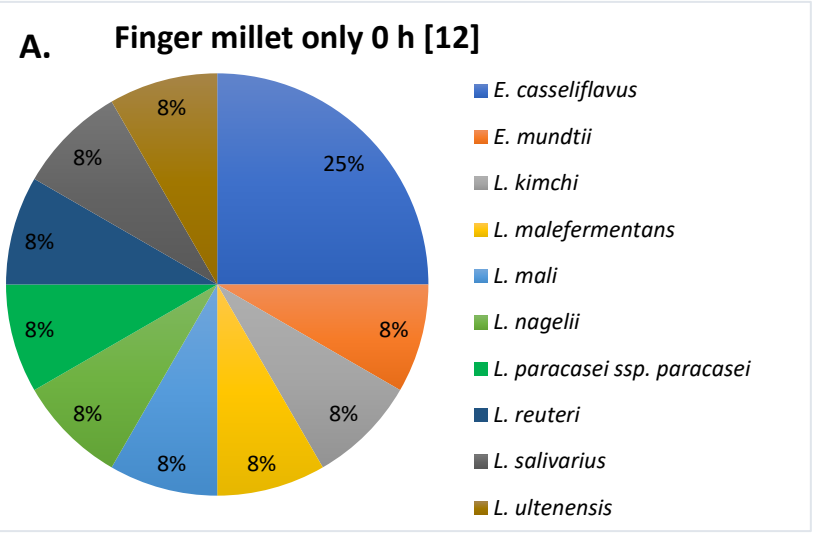
Figure 3.6. Effect of starch source and fermentation time on yeast count of finger millet-based slurries. Letter C represents finger millet only slurry (control), M represents finger millet and maize starch slurry, and R represents finger millet and rice slurry. Values are the mean (± SD) of 3×2 replications (n=6). Different superscripts in the same time category indicate significant differences

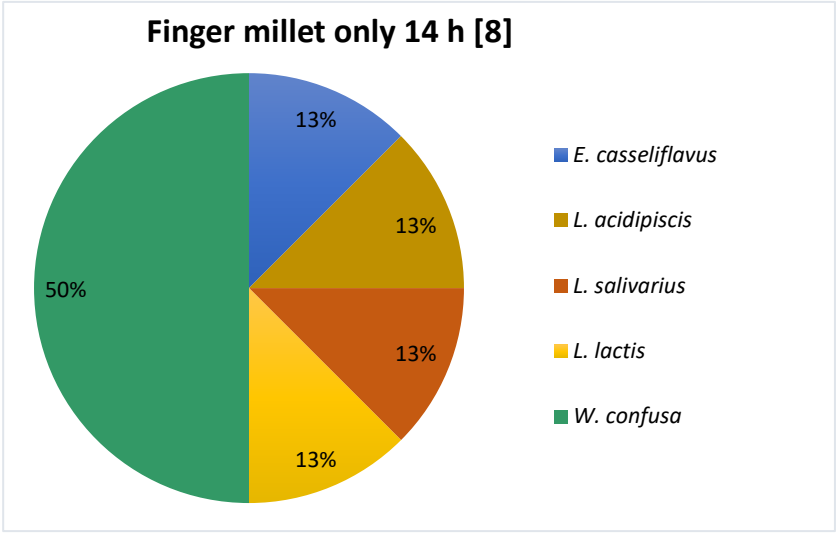
Figure 3.6 shows the yeast counts of finger millet-based beverages at the onset and end of spontaneous fermentation. The results show that the start of fermentation was dominated by yeast whose population increased from 5.78 ± 0.47 – 6.08 ± 0.20 to 9.05 ± 0.39 – 9.36 ± 0.55 $\log_{10}$ cfu/ml after 14 h of the fermentation process. Overall, there was no difference in yeast counts between the three fermentation treatments at 0 h and 14 h. Therefore, the addition of exogenous starch slurry, whether maize or rice, did not affect yeast counts both at the onset and the end of fermentation. A similar trend in yeast proliferation was observed by Houngbédji *et al.* (2018) for *mawè*, a West African cereal-based dough prepared traditionally by spontaneous fermentation. In their study, the type of cereal (sorghum or maize) used did not affect yeast counts throughout fermentation. Generally, yeasts have been associated with LAB during the fermentation of cereal-based food products. This is because LAB create a high acid environment that favours the proliferation of certain yeast species such as Candida. The co-metabolism of certain yeast species with LAB has been reported to be desirable for adequate fermentation of certain traditional African food which gives them peculiar physical attributes (Nout & Motarjemi, 1997; Greppi, Rantisou, Padonou, Hounhouigan, Jespersen, Jakobsen & Cocolin, 2013; Houngbédji *et al.*, 2018).

3.1.3.5 Microbial identification by MALDI-TOF MS

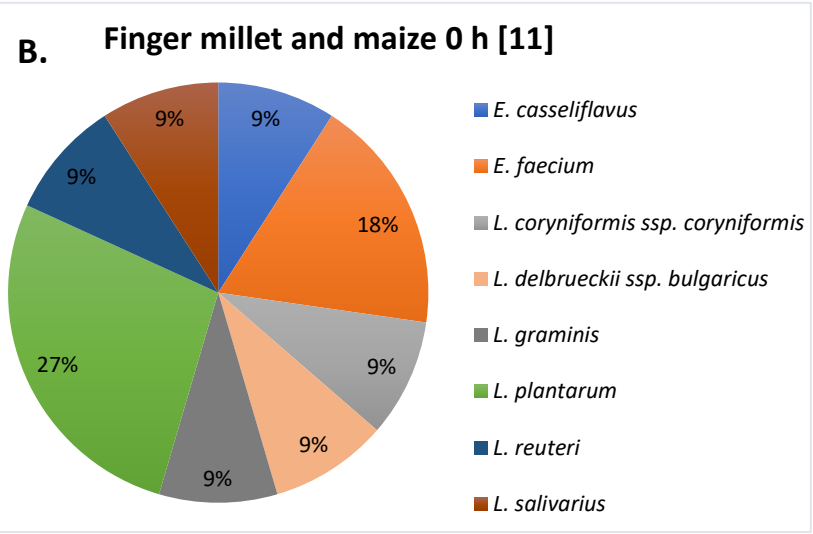
The initial identification of isolates indicated that only 57 spectra, constituting about 63.33% of the total isolates (90), were successfully identified as LAB. Summative, 24.56% of the isolates corresponded to *W. confusa*, 17.54% to *E. casseliflavus*, 5.26% to *L. plantarum*, 5.26% *L. amylophilus*, 5.26% *L. paracasei* ssp. *paracasei*, 3.51% *E. faecium* and finally 1.94% which were other LAB species that occurred at less than 2% abundance (Figure 3.7). The succession dynamics based on the preliminary characterisation of LAB are discussed below.

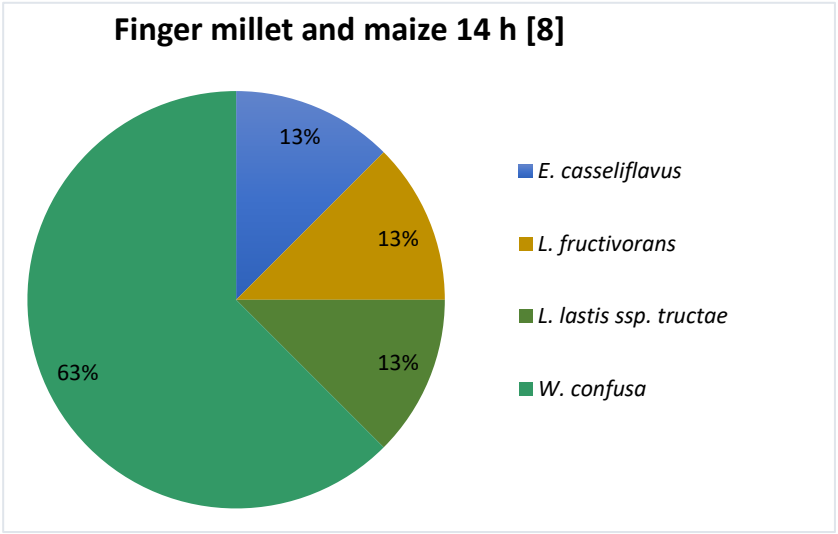
A.

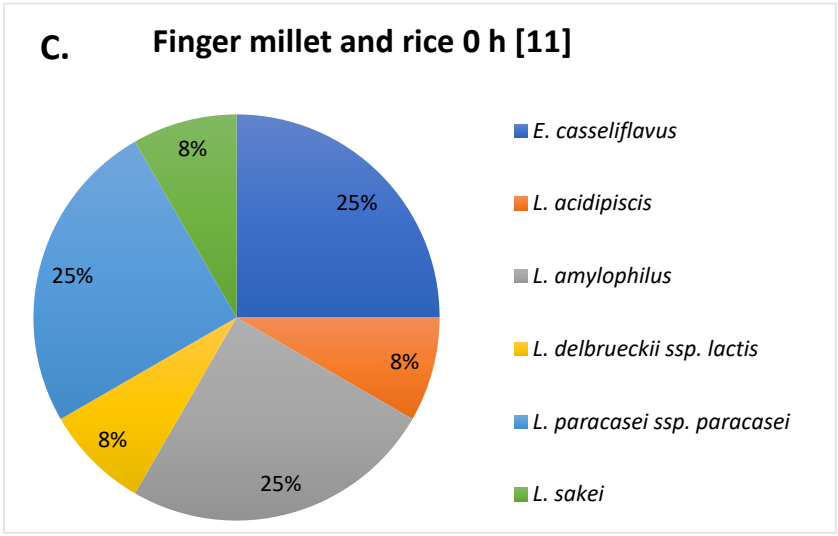




B.







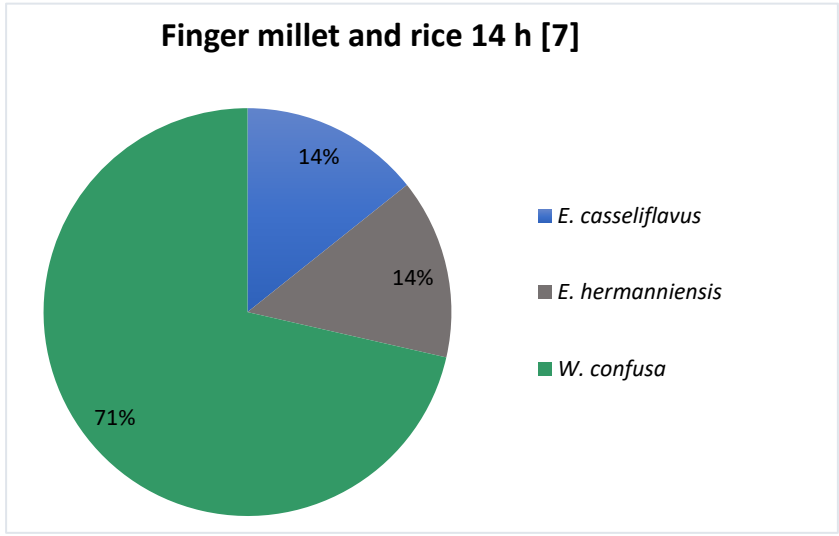


Figure 3.7. LAB species diversity of finger millet-based beverages. A. finger millet only, B. finger millet and maize starch, C. finger millet and rice starch slurry at 0 h and 14 h of fermentation. Numbers in brackets indicate the number of LAB isolates included in the calculations

The succession of LAB species during the fermentation of finger millet-based slurries is shown in Figure 3.7. The results of the analysis show that the fermentations were dominated by bacterial species belonging to the genera *Enterococcus*, *Lactobacillus*, *Lactococcus*, and *Leuconostocaceae*. *E. casseliflavus* was the most dominant species at the start of fermentation in the finger millet only formulation together with *E. faecium*, *L. plantarum,* and *L. amylophilus,* and *L. paracasei* ssp. *paracasei* in both formulations with added starch slurry (M and R, respectively). There were, however, other LAB species of the genera *Lactobacillus* at low abundance which are often associated with cereal fermentation. The identified species were *L. mali*, *L. delbrueckii* ssp. *bulgaricus*, *L. acidophilus*, *L. plantarum*, *L. salivarius*, *L. malefermentans*, *L. coryniformis* ssp. *coryniformis*, *L. kimchi*, *L. nagelii*, and *L. reuteri*. Isolated at the start (0 h) and end (14 h) of fermentation for all the treatments was *E. casseliflavus* while *W.* confusa, *L. lastis*, *L. fructivorans,* and *E. hermanniensis* were isolated only after 14 h of fermentation. Also detected after 14 h of fermentation were *L. acidipiscis*, *L. lactis,* and *L. salivarius* in fermented beverage C, and *L. fructivorans* and *L. lastis* ssp. *tructae* in sample M. From these results it can be concluded that the raw materials used for fermentation had an influence on the type of LAB species both at the start (0 h) and end (14 h). Some species such as *E. casseliflavus* and *W. confusa* were, however, common in all three treatments. The analysis, therefore, revealed that spontaneous fermentation of finger millet-based beverages was dominated by LAB. From a gut health perspective, the dominance of *Weissella* in this fermentation study is of significance due to the health benefits attached to the strain particularly *W. confusa* which is described as one of the highest producers of microbial exo-polysaccharides (EPSs). EPSs are essential bioactive compounds recognised for their therapeutic properties such antioxidant, cholesterol lowering, antidiabetic properties and others. This, therefore, suggests the potential of *W. confusa* as a probiotic (Fessard & Remize, 2017; Nampoothiri, Beena, Vasanthakumari and Ismail, 2017).

During the fermentation of *Suanzhou*, a traditional Chinese gruel prepared from proso millet, millet, and small amounts of rice, the microbial analysis indicated that LAB were one of the key microbes involved in the fermentation of the product (Qin, Sun, Pan, Qiao & Yang, 2016). In another study by Houngbédji *et al.* (2018) five different LAB species were identified in commercial and homemade maize and sorghum-based fermented doughs namely *L. fermentum*, *L. plantarum*, *P. acidilactici*, *W. confusa*, and *P. pentosaceus*. However, in this work, a higher LAB diversity was observed compared to the former study. These findings support the use of

LAB as starter cultures in the cereal fermentation industry seeing that they are highly associated with the fermentation of most cereal-based products (Mugula *et al.*, 2003; Blandino *et al.*, 2003; Moodley, 2015).

3.1.3.4 Impact of starch source on the dynamics of microbial composition during fermentation through 16S rRNA gene amplicon sequencing

To better understand the community structure of fermenting microbiota in spontaneous fermentation of finger millet with added maize and rice starch, the bacterial DNA extracts were analysed by 16S rRNA gene amplicon sequencing. Amplification and sequencing of the 16S rRNA gene V4 variable region yielded a total of 5,585,665 multiplexed amplicon sequences. To reduce sequencing errors and dereplicate sequences, low-quality reads were denoised, and chimeras were trimmed to 2,297,080 using the DADA2 pipeline available in QIIME2 version 2019.7 (Callahan *et al.*, 2016). The definition of Operational Taxonomic Units (OTUs) was done by clustering at a 3% divergence (97% similarity score).

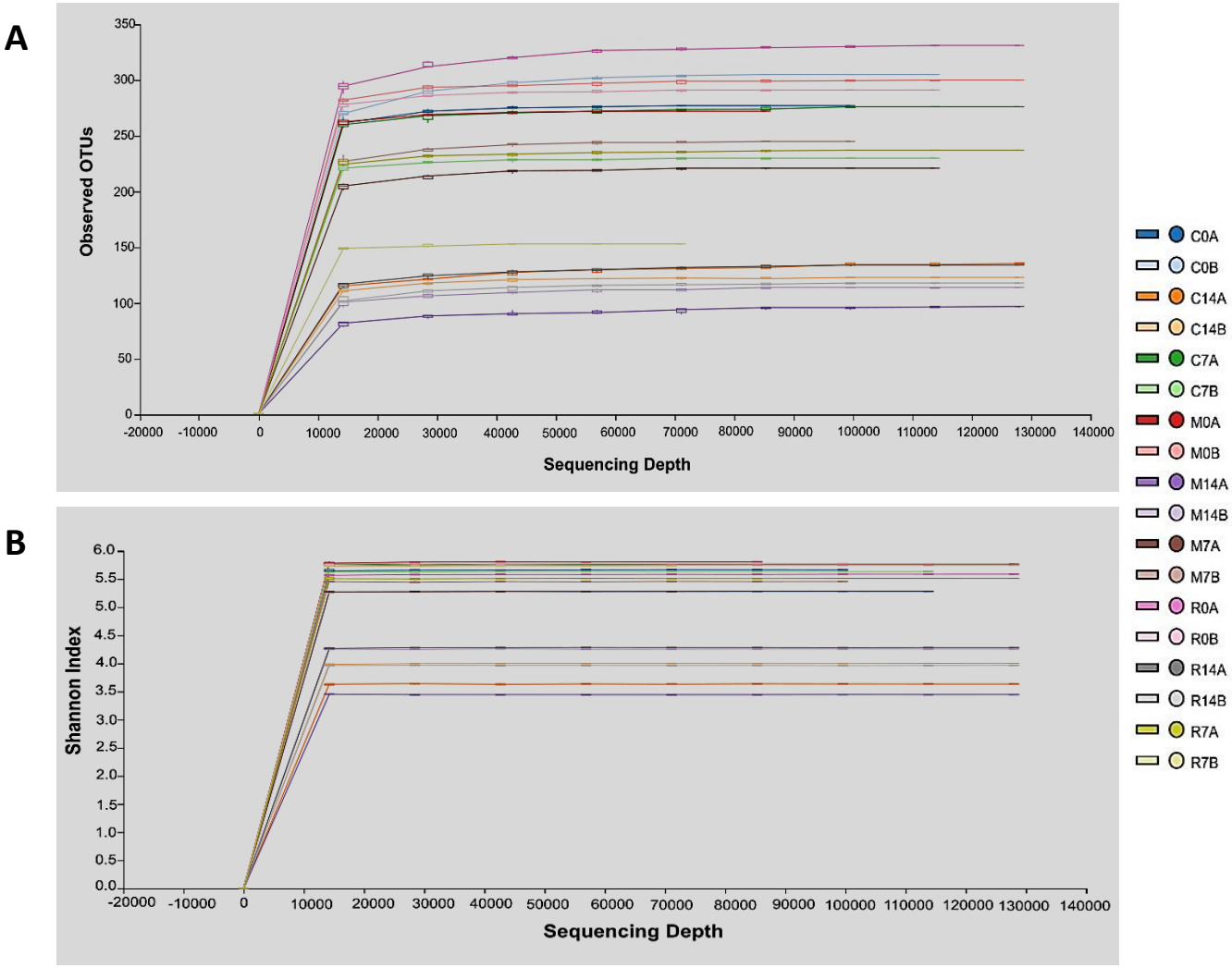


Figure 3.8. Observed OTUs (A) and Shannon (B) rarefaction plots for the fermentation treatments at 0, 7 and 14 h of fermentation. C- finger millet (control), M- finger millet and maize starch slurry, R- finger millet and rice starch slurry

Based on the median non-chimeric sequences (128,987) for all the samples, the sequencing depth was rarefied to 120,000 to ensure wider coverage of bacterial diversities. The rarefaction plots (Figure 3.8) give a clear indication that paired-end sequences were well sampled hence OTUs and Shannon Index rarefaction curves reached a plateau.

3.1.3.5 Variations in the taxonomic profile of the microbiota during the fermentation process

Based on the QIIME taxonomic classification data, the phyla Proteobacteria and Firmicutes were dominant throughout the fermentation process, both comprising about 80-90% of the bacterial population in all the fermentation samples (Figure 3.9A). *Sphingomonas* and *Rhizobium* were the dominant genera at the onset of fermentation in all the samples and were succeeded by the genera *Weissella* (from 2.06% to 64.31%), phylum "Firmicutes" which were the most abundant after 14 h of fermentation (Figure 3.9B). *Sphingomonas, Enterococcus, Staphylococcus, Enterobacter*, and *Rhizobium* are genera of bacteria commonly found in crops and fermented foods (Ayodeji, Piccirillo, Ferraro, Moreira, Obadina, Sanni & Pintado, 2017). In a typical spontaneous fermentation process with plant-based foods, the predominance of environmental or crop-specific symbiotic bacteria is common in the first 7 to 12 h of the process (Resende *et al.*, 2018). However, in this study, these bacteria were reduced or not detected at the end of fermentation with the dominance of LAB. In particular, the increasing abundance of LAB, genera *Weissella*, during the process might be the influencing factor lowering the pH of the medium. Interestingly, many other studies supported the intense growth of *Weissella* at the end of the fermentation in indigenous beverages (Mugula *et al.*, 2003; Coulin, Farah, Assanvo, Spillmann & Puhan, 2006; Freire, Ramos, de Almeida, Duarte & Schwan, 2014; Elizaquível, Pérez-Cataluña, Yépez, Aristimuño, Jiménez, Cocconcelli, Vignolo & Aznar, 2015).

Among the prevailing trends in fermenting microbiota, (i) the control group (fermented finger millet only) had the highest *Weissella* concentration (64.31%) followed by the rice slurry formulation (60.91%) and finally the maize formulation (56.1%); (ii) following *Weissella*, the genera *Enterococcus* increased in abundance throughout fermentation from 5.25% to 22.28% in all the treatments; (iii) the formulation with maize starch showed the highest *Lactococcus* activity (11.16%) after 14 h of fermentation; (iv) At the onset, the fermentation process was dominated by the phylum Proteobacteria (60.67%) which decreased gradually to 11.63% in the control sample. This is due to the increase in the relative abundance of LAB, as highlighted above. Likewise, in a study exclusive for starch fermentation, bacteria belonging to the phylum

Firmicutes were identified as dominant carbohydrate fermenters that facilitate the metabolism of biomolecular structures in the fermentation medium (Payling, Fraser, Loveday, Sims, Roy & McNabb, 2020).

In line with these results, another microbial dynamics study revealed that the acidification of slurries is linked to the growth of LAB which could also be the reason behind the antimicrobial role against pathogenic bacteria in the food fermentation process (Castellano, Belfiore, Fadda & Vignolo, 2008; Fessard & Remize, 2017). In another study by Diaz, Kellingray, Akinyemi, Adefiranye, Olaonipekun, Bayili, Ibezim, du Plessis, Houngbédji and Kamya (2019), a lower relative abundance of lactobacilli was observed in cereal-based samples fermented at laboratory scale compared to samples fermented under household conditions. The fermenting microbes were dominated by the genera *Lactobacillales* particularly *Lactobacillus*, *Weissell*a and *Streptococcus*. The differences in bacterial diversity can be linked to production conditions, where laboratory fermentation is often done under sterile conditions contrary to artisanal production where back slopping of microorganisms establishes consistent fermentation microflora (Nout & Motarjemi, 1997).

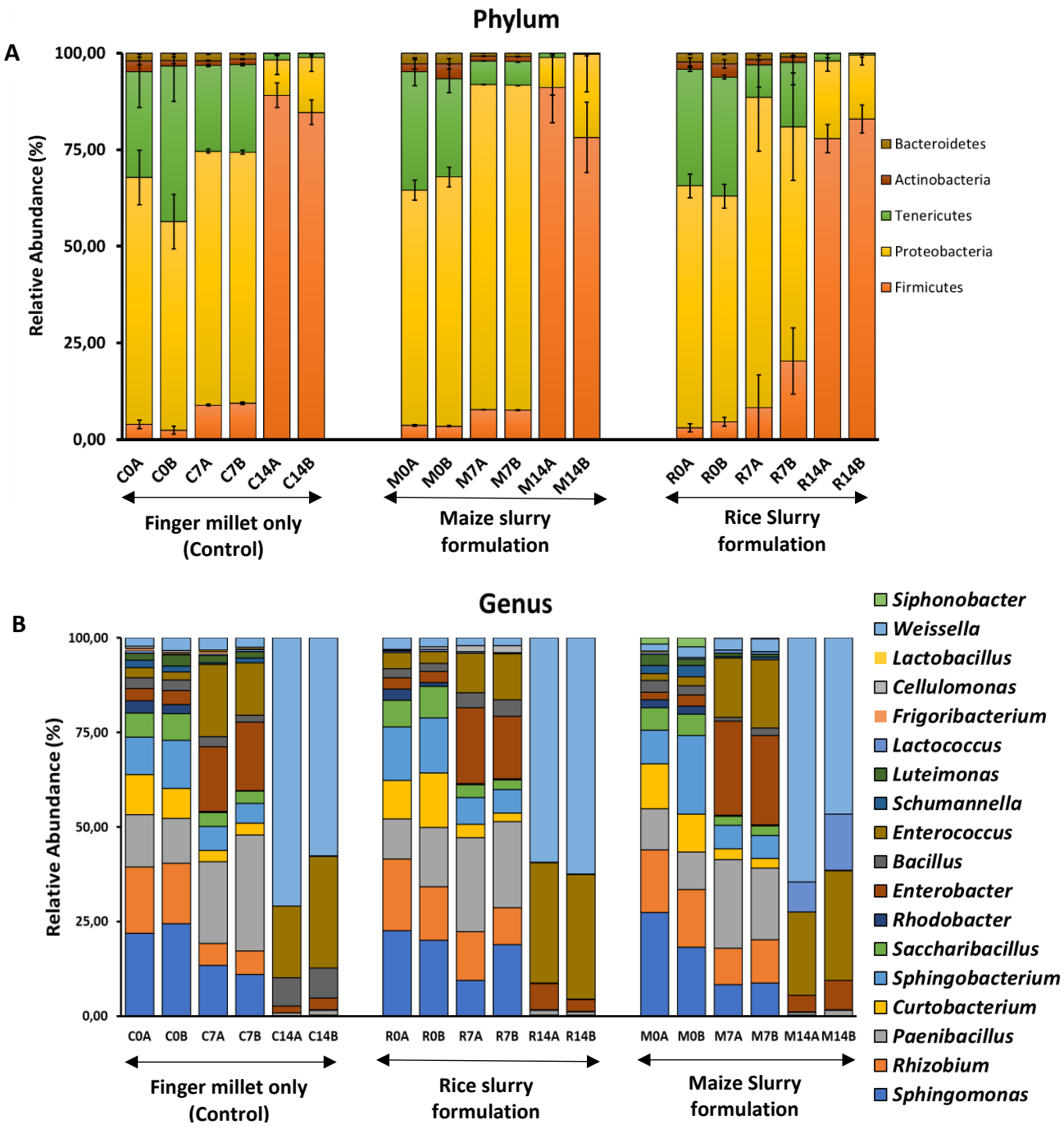


Figure 3.9. The relative abundance of bacterial communities at phylum (A) and genus (B) level for the different formulations at different time intervals (0, 7 and 14 h)

3.1.3.6 Analysis of compositional variance (ANCOM)

A differential abundance test (ANCOM) was performed through QIIME2 software to test the hypothesis that the abundance of microbiome composition is different between the groups. Although variations are visible through bar graphs in the percentage relative abundance of taxa at the phylum and genus level between the samples (Figure 3.9), the ANCOM test results (Figure 3.10) indicated no differences in the relative abundance of taxa between the fermentation groups.

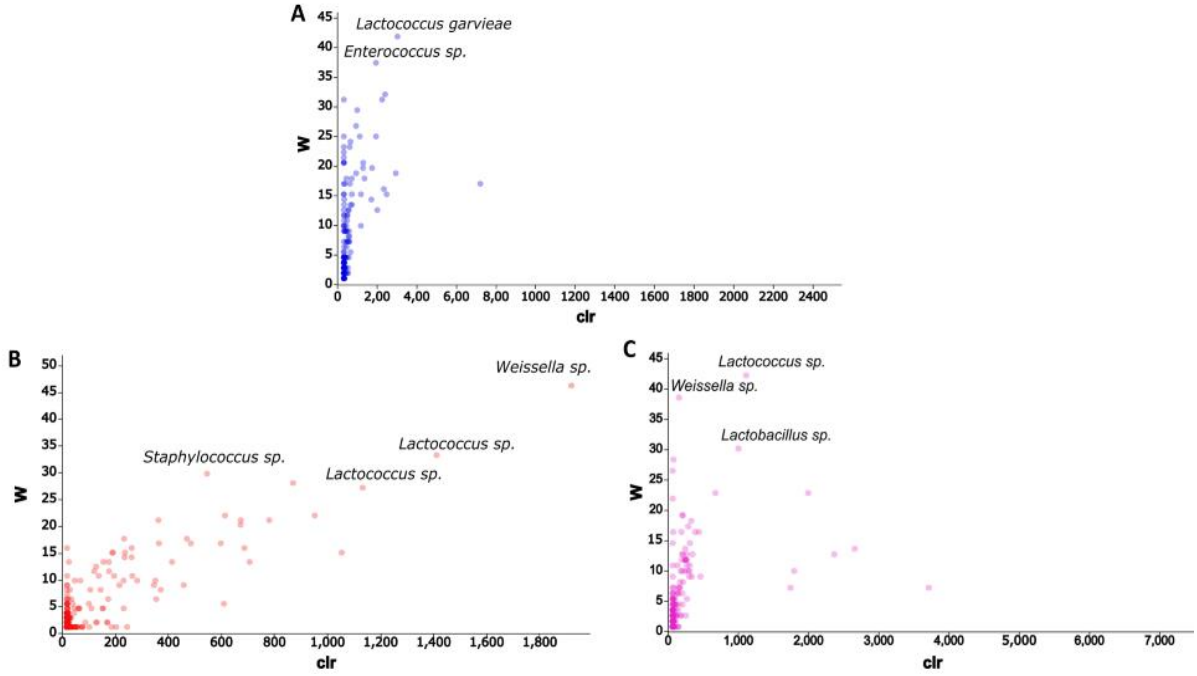


Figure 3.10. ANCOM differential abundance volcano plot. A. Control (Finger millet only), B. Finger millet and maize starch, and C. Finger millet and rice starch. The centered log-ratio (clr) transformed OTU table at the genus level with 0 values adjusted to 1 was used. Few species which reject the null hypothesis (W) more than 30 times are labelled. All groups have an equal average abundance of species. The W values (the number of times the null hypothesis is rejected for a given species) of LAB, such as *Enterococcus* sp. were above 30 in the comparison of relative abundance of taxa among the fermentation groups.

The high abundance of *Weissella* in spontaneously fermented finger millet-based beverages highlights its important role in the fermentation process and raises a hypothesis on its potential use as a starter culture in cereal-based fermentations. The 16S rRNA amplicon sequence analysis results revealed a more reliable and accurate taxonomic classification in terms of

differentiation of LAB species (Böhme, Fernández-No, Pazos, Gallardo, Barros-Velázquez, Cañas & Calo-Mata, 2013; Lv, Jia, Li, Chen, Chen, Liu, Chen, Rao & Ni, 2016).

3.1.3.7 Diversity analysis of group microbiota at different fermentation time intervals

Table 3.4. A statistical table showing between-treatment effects

Alpha- diversity	**H**	**p-value**	**q-value**
Evenness- group significance			
C vs M @ 0 H	2.4	0.12	0.36
C vs R @ 0 H	0.6	0.44	0.44
M vs R at 0 H	0.6	0.44	0.44
C vs M @ 7 H	2.4	0.12	0.18
C vs R @ 7 H	0.0	1.0	1.0
M vs R at 7 H	2.4	0.12	0.18
C vs M @ 14 H	0.6	0.44	0.66
C vs R @ 14 H	2.4	0.12	0.36
M vs R at 14 H	0.0	1.0	1.0
Faith's phylogenetic diversity			
C vs M @ 0 H	0.6	0.44	0.66
C vs R @ 0 H	0.6	0.44	0.66
M vs R at 0 H	0.0	1.0	1.0
C vs M @ 7 H	0.6	0.44	1.0
C vs R @ 7 H	0.0	1.0	1.0
M vs R at 7 H	0.0	1.0	1.0
C vs M @ 14 H	2.4	0.12	0.18
C vs R @ 14 H	0.0	1.0	1.0
M vs R at 14 H	2.4	0.12	0.18
Shannon's diversity			
C vs M @ 0 H	2.4	0.12	0.36
C vs R @ 0 H	0.6	0.44	0.44
M vs R at 0 H	0.6	0.44	0.44
C vs M @ 7 H	2.4	0.12	0.18

C vs R @ 7 H	0.6	0.44	0.44
M vs R at 7 H	2.4	0.12	0.18
C vs M @ 14 H	0.0	1.0	1.0
C vs R @ 14 H	0.6	0.44	0.66
M vs R at 14 H	0.6	0.44	0.66
Beta- diversity	**Pseudo-F**	**p-value**	**q-value**
Bray- Curtis distance			
C vs M @ 0 H	0.61	1.0	1.0
C vs R @ 0 H	0.68	1.0	1.0
M vs R at 0 H	0.86	1.0	1.0
C vs M @ 7 H	14.91	0.36	0.36
C vs R @ 7 H	1.04	0.36	0.36
M vs R at 7 H	1.72	0.31	0.36
C vs M @ 14 H	0.56	0.64	0.95
C vs R @ 14 H	1.62	0.33	0.95
M vs R at 14 H	0.16	1.0	1.0
Jaccard distance			
C vs M @ 0 H	0.99	0.68	1.0
C vs R @ 0 H	1.0	0.34	1.0
M vs R at 0 H	0.97	1.0	1.0
C vs M @ 7 H	1.04	0.66	0.66
C vs R @ 7 H	0.98	0.66	0.66
M vs R at 7 H	1.10	0.30	0.66
C vs M @ 14 H	0.98	0.67	0.67
C vs R @ 14 H	1.04	0.34	0.67
M vs R at 14 H	0.98	0.65	0.67

The letter C represents finger millet only (control), M represents finger millet and maize starch slurry, and R represents finger millet and rice starch slurry. The “H” value indicates the test statistical value in the Kruskal-Wallis test, the pseudo-F value indicates the test statistical value in the PERMANOVA test, and the “p” value indicates the statistical significance among the study groups. No differences were observed between the fermentation groups (C, M, and R) at 0, 7 and 14 h of fermentation ($p > 0.05$) (n=2).

Table 3.4 shows the diversity analysis and statistical outcomes for between-treatment interactions at the three levels of sampling time (0, 7, and 14 h).

3.1.3.7.1 Alpha Diversity

The alpha diversity indices were calculated to evaluate microbial richness, evenness, and diversity within the fermentation groups based on the starch source. The metrices such as Shannon's diversity estimation, Faith's phylogenetic diversity, and Pielou's evenness estimation were estimated based on microbial community variations. The box and whisker plots (Figure 3.11) showed that there were no differences in alpha diversity indexes during the fermentation process of samples with added starch in comparison with the control. This is indicative that there were no rare species identified between the three formulations (C, M, and R) at the different stages of the fermentation process. More so, similarities were observed in the species frequencies between the fermentation groups hence no relative differences in the abundance of species within the population were identified based on the starch source (rice starch and maize starch). There was, however, a statistically significant difference ($p < 0.05$) in the population of microflora within the three groups with an increase in fermentation time as highlighted above (Table 3.3). The insignificant OTU diversity observed after fermenting for 14 h further highlights the selective pressure (acidity) on the community of organisms that favours only acid-adapted species such as LAB (Gaffa & Gaffa, 2004; Ezekiel, Ayeni, Ezeokoli, Sulyok, van Wyk, Oyedele, Akinyemi, Chibuzor-Onyema, Adeleke & Nwangburuka, 2019).

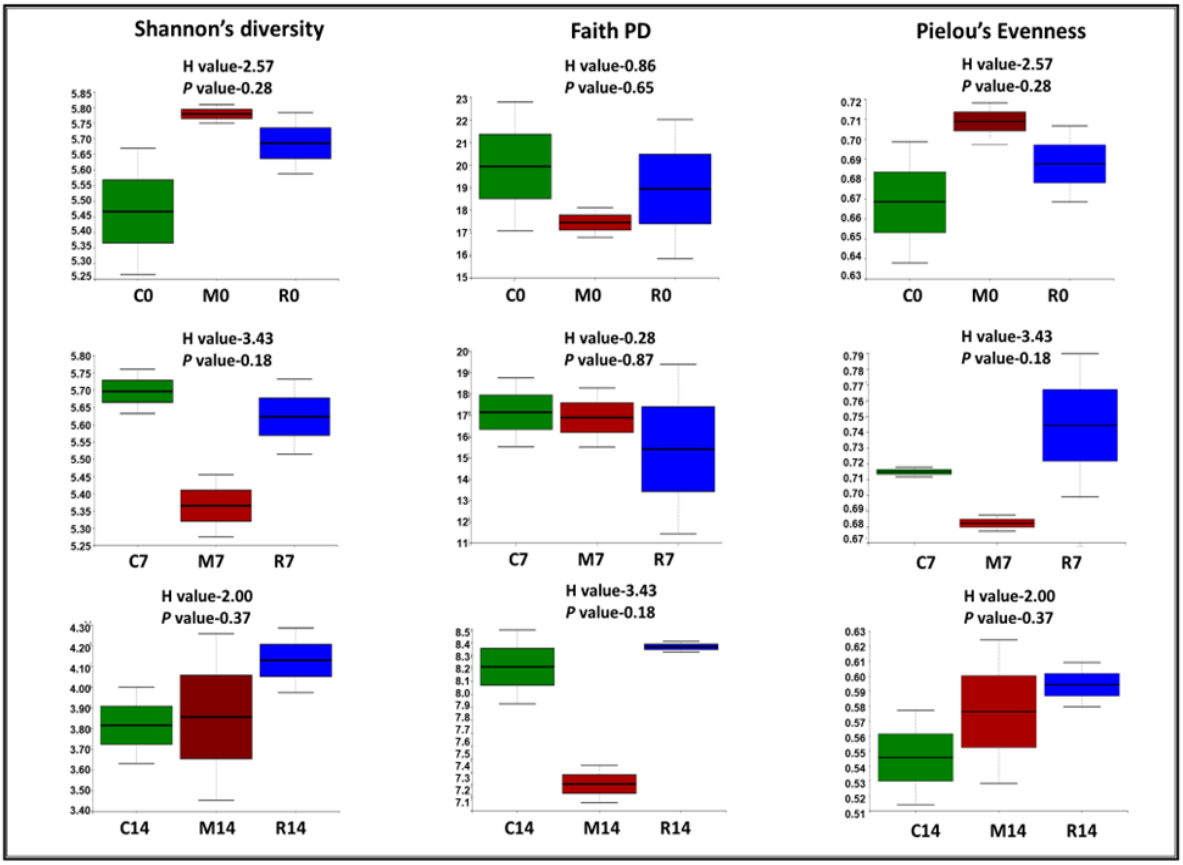


Figure 3.11. Box and whisker plots of the variance in Shannon, Faith Phylogenetic diversity and Pielou's evenness of bacterial communities, as obtained by QIIME-2 analysis. The "H" value indicates the test statistical value in the Kruskal-Wallis test and "P" value indicates the statistical significance among the study groups

3.1.3.7.2 Beta diversity

To understand the role of starch source (treatment) and the influence of fermentation time on microbiota, beta-diversity using the UniFrac distance matrix was computed. The beta diversity calculates the microbial diversity between the samples, unlike the diversity within the samples for alpha diversity (Krishnamoorthy, Coetzee, Kruger, Potgieter & Buys, 2020). The one-way permutational multivariate analysis of variance test through weighed, unweighted, Jaccard index, and bray Curtis indices showed no difference in the study groups at a 95% confidence interval. The principal coordinate analysis and the coordinate values are shown in Figure 3.12. Despite the impact of starch source on microbial dysbiosis not being observed in this study, there was a significant increase ($p < 0.05$) in microbial communities after 7 h of fermentation in all the study groups.

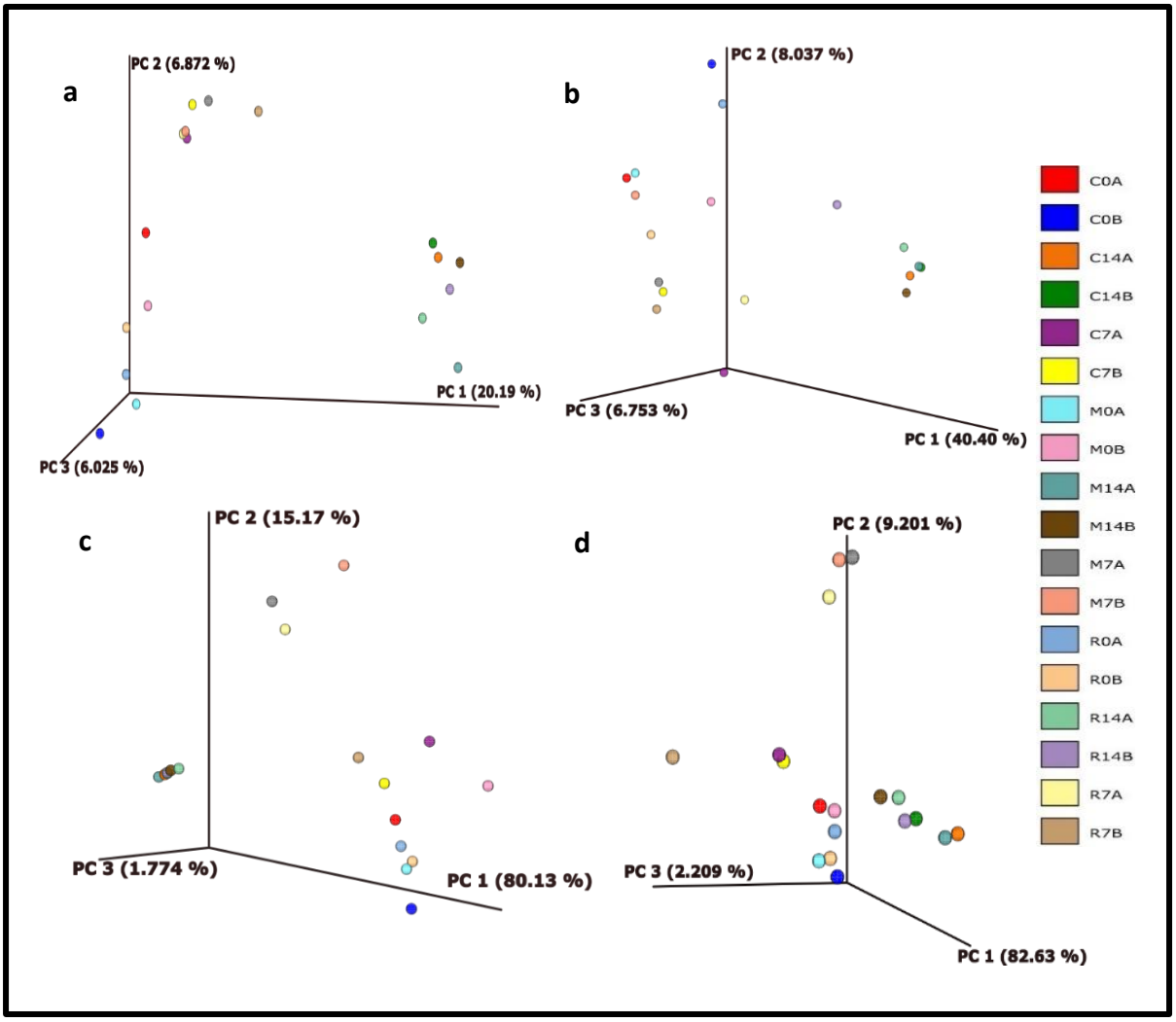


Figure 3.12. Principal coordinates analysis (PCoA) with a) Jaccard, b) unweighted c) weighted d) Bray Curtis Unifrac distance plots grouped according to treatment and fermentation time. The values in the axis represent the percentage variation within the groups

3.1.4 Conclusion

The spontaneous and traditional solid-state fermentation of cereals is a dynamic process characterised by the activity of diverse microbial populations linked to the local fermentation environmental conditions (Li, Lin, Liu, Wang & Luo, 2016). In this finger millet fermentation study, various factors such as fermentation temperature (28 °C), acidity, and pH of the medium, which are directly related, have determined bacterial succession. The decrease in pH with an increase in titratable acidity (Figure 3.3) demonstrates the growth of microbes in this open-environment particularly yeast and LAB which dominate at the end of fermentation. In contrast, a combination of crop-specific bacteria commonly isolated in maize, finger millet and

rice and other potentially pathogenic bacteria belonging to the genera *Sphingomonas, Enterococcus, Staphylococcus, Enterobacter*, and *Rhizobium* dominate the fermentation process in the beginning but later out-competed by yeast and then by LAB in this study.

Regarding the addition of starch source (maize and rice) on the taxonomic profile change in the spontaneous fermentation process, no differences are observed (Figure 3.8). Similar and successional dynamics of microbial composition are exhibited during the fermentation process at phylum as well as at the genus level. In the ANCOM analysis, which is used for spotting the abundant bacterial genera influencing a difference among the groups, several LAB (with "W" values of more than 30) such as *Weissella* sp. and *Enterococcus* sp. were seen in the volcano plot but again fail to make a difference between the groups. In the bacterial diversity analysis, the alpha diversity (microbiota richness/ evenness within the sample) and beta diversity (microbiota richness/ evenness between the samples) showed no differences ($p > 0.05$) in all the categorized groups (Figure 3.11 and Figure 3.12) except fermentation time. However, one of the interesting findings of this study is the dominance of *Weissella* ssp. (LAB) in the fermentation process for all three fermentation treatments (C, R and M). This study, therefore, highlights the potential of *Weissella* ssp. as important commercial starter cultures for large-scale finger millet-based fermentations.

Since maize is an important staple in the African region, it is worth emphasizing that fermented finger millet-based beverages with added maize starch slurry could be prepared and consumed in comparison to Indian *ambali*. This highlights that interchanging the rice starch slurry for maize starch will not interfere with the overall microbial communities which participate in the characteristics of the final product. Overall, the importance of the microbiome study report is meant not only to unravel the fermentation science behind the addition of starch but also to encourage the appreciation of the practice of preparing finger millet-based slurries with added maize and/ or rice as additional starch sources during the fermentation process.

3.2 Nutritional and antioxidant properties of finger millet-based beverages as influenced by starch source during lactic acid fermentation

Abstract

The effect of adding exogenous maize (M- finger millet and maize slurry) and rice (R- finger millet and rice slurry) starch slurries on the nutritional and bioactive properties of finger millet-based beverages was evaluated. Beverages were fermented for 14 h following the spontaneous fermentation process, cooked and analysed for phytate content, mineral bioaccessibility, protein quality (protein content, in vitro protein digestibility, reactive lysine), total phenolic content (TPC), and antioxidant activity (AA). Total starch content and microstructure of starch slurries were also determined. The microstructure studies revealed the presence of partially gelatinised starch in both maize and rice slurries. Beverage samples with added starch showed significantly ($p < 0.05$) lower overall phytate content and significantly ($p < 0.05$) higher overall bioaccessible iron and zinc compared to finger millet only samples. Protein quality [*in vitro* protein digestibility (IVPD) and reactive lysine content], TPC and AA were not significantly ($p > 0.05$) altered by the starch source as an independent variable. Fermentation was effective in reducing the phytate levels of samples, with or without added starch, followed by an increase and decrease in bioaccessible zinc and iron, respectively as well as an increase in TPC and AA. While cooked samples had a lower percentage IVPD compared to uncooked samples, fermentation was effective in alleviating these negative effects of cooking. While fermentation did not affect reactive lysine content, it was significantly reduced by cooking. Overall, this study shows that the addition of an exogenous starch source in combination with fermentation can be used to improve mineral bioaccessibility, TPC, and AA of finger millet-based beverages while maintaining appreciable protein quality. These soured beverages can contribute to the alleviation of malnutrition and non-communicable diseases especially in low-income communities of Sub-Saharan Africa (SSA).

Keywords: finger millet, maize, rice, fermentation, cooking, mineral bioaccessibility, *in vitro* protein digestibility (IVPD), reactive lysine, phytic acid, total phenolic content (TPC), antioxidant activity (AA).

3.2.1 Introduction

Finger millet (*Eleucine coracana* (L.) Gaertn.) is a drought-tolerant crop that is cultivated in different parts of Africa and Asia majorly for food (Taylor & Emmambux, 2008). It is processed into flour and used in the preparation of cakes, bread, pastry products and, porridges, and non-alcoholic beverages which are beneficial weaning foods for infants especially in low-income communities (Antony Ceasar, Maharajan, Ajeesh Krishna, Ramakrishnan, Victor Roch, Satish & Ignacimuthu, 2018).

Although finger millet has a fair amount of proteins and minerals compared to other cereals such as wheat, rice, and sorghum (Sharma, Jamra, Singh, Sood & Kumar, 2017), its nutritional quality is relatively poor due to deficiency of essential amino acids such as lysine and abundance of antinutritional factors such as phytate, which reduce the digestibility of proteins and availability of minerals *in vitro* (Kumar *et al.*, 2010). Hence the prevalence of protein malnutrition and mineral deficiency especially in Africa where finger millet forms an essential part of the diet (Ramakrishnan, 2002; Temba, Njobeh, Adebo, Olugbile & Kayitesi, 2016). Aside from this, the consumption of finger millet has been associated with reduced risk of non-communicable diseases (NCDs) such as certain types of cancers, diabetes mellitus, and coronary heart disease (Kumar *et al.*, 2010; Siwela, Taylor, de Milliano & Duodu, 2010). This is because finger millet is rich in bioactive active constituents such as phenolic compounds which are considered to have health-promoting properties (Adebo & Gabriela Medina-Meza, 2020).

Spontaneous fermentation is an ancient traditional processing techniques that is used to transform cereal grains, including finger millet, into value-added food products with improved nutritional and health-promoting properties (Adebo *et al.*, 2018; Adebo & Gabriela Medina-Meza, 2020). Antony and Chandra (1998) reported improvements in soluble protein and *in vitro* protein digestibility as well as enhanced mineral availability with a concomitant decrease in phytate content after fermentation of finger millet. Improvements in protein quality during lactic acid fermentation are often linked to structural modification of storage proteins at low pH which makes proteins more accessible to proteolytic enzymes (Taylor & Taylor, 2002), whereas an increase in bioaccessible minerals is generally associated with the degradation of phytate at low pH by microbial and grain phytases which reduces its ability to chelate divalent minerals (Feil, 2001).

The potential of fermentation to improve the absorption mechanism of cell wall-bound phenolic compounds consequently the antioxidant activity of plant-based food has been reviewed (Acosta-Estrada, Gutiérrez-Uribe & Serna-Saldívar, 2014). In a study by Gabaza *et al.* (2016) on finger millet porridge and intermediate products, fermentation caused an increase in soluble phenolic compounds which suggested microbial enzymes degradation of polymerised phenolic compounds or cell wall-bound phenolic compounds into forms which can be easily extracted (free/unbound forms of the phenolics). Phenolic compounds possess antioxidant properties, hence an increase in their levels during fermentation is beneficial for improved human health (Adom & Liu, 2002).

During the preparation of different of cereal-based products, the addition of extra sources of starch during the fermentation process is a common practice. Examples of such products include *amahewu* and *ambali* (Gadaga *et al.*, 1999; Blandino *et al.*, 2003; Ray *et al.*, 2016). During *amahewu* preparation, corn/maize meal is used as the major fermentation substrate, however, sorghum, millet malt, or wheat flour are added before it is left to ferment (Gadaga *et al.*, 1999). On the other hand, *ambali* is prepared from a mixture of finger millet slurry and partially cooked rice (Ray *et al.*, 2016). While no single reason can be attached to this practice, one obvious reason is that of supplementing the starch content of the fermentation medium and also introducing microbial flora and enzymes, where malt flour is concerned, to initiate lactic acid fermentation. Ray *et al.* (2016) suggest that the co-fermentation of cereals such as rice with other cereals can improve the overall nutritional capacity and therapeutic potential of cereal-based foods. In this context, the implications of adding different sources of starch during fermentation of cereal-based products on the properties of the final product need to be investigated. Therefore, this study aims to investigate the effect of adding exogenous maize and rice starch slurries on the nutritional and bioactive properties of finger millet-based products prepared by spontaneous fermentation.

3.2.2 Materials and methods

3.2.2.1 Materials

The cereal grains used, and preparation of flours and the rice and maize starch slurries were as described previously in sections 3.1.2.1.1 and 3.1.2.1.2.

3.2.2.2 Preparation of finger millet-based beverages through the spontaneous fermentation process

This was done following the experimental procedure described in section 3.1.2.1.3. Following fermentation, slurries were diluted with distilled water (1:1, v/v), and boiled for 15 min with stirring at regular intervals. Gruels were freeze-dried, crushed to a powder, and stored at -4 °C for biochemical analysis. The experimental plan is illustrated in Figure 3.13. Sample codes and descriptions are tabulated in Table 3.1.

3.2.2.2 Analytical methods

3.2.2.2.1 Total starch

Total starch content of the maize and rice starch slurries was determined using the Megazyme Total starch enzyme assay kit procedure adapted from Mccleary, Gibson and Mugford (1997). The method is based on the use of thermostable α-amylase which hydrolyses starch into soluble maltodextrins and amyloglucosidase (AMG) which further hydrolyses maltodextrins to D-glucose. Briefly, accurately weighed freeze-dried powder of the maize and rice starch slurries (100 mg) were mixed with 2 M KOH and stirred continuously for 20 min in an ice water bath to pre-dissolve retrograded starch followed by neutralisation with 8 mL of 1.2 M sodium acetate buffer (pH 3.8). Immediately, 0.1 mL thermostable α- amylase and 0.1 mL AMG were added to each tube and incubated at 50ºC with intermittent mixing for 30 min. At the end of incubation, the volume of the digest was brought to 100 mL with distilled water and a homogenous aliquot of the solution centrifuged at 1 800 x *g* for 10 min to precipitate the enzymes and other non-starch residues. Aliquots (0.1 mL) of the supernatant were reacted with 3 mL of glucose oxidase peroxidase [GOPOD Reagent enzymes] (Megazyme International Ireland, Wicklow, Ireland) at 50ºC for 20 min. The amount of D-glucose released during starch digestion was colorimetrically measured at 510 nm against the reagent blank. Regular maize starch containing 93% starch and D-glucose solution (Megazyme International Ireland, Wicklow, Ireland) were used as standards.

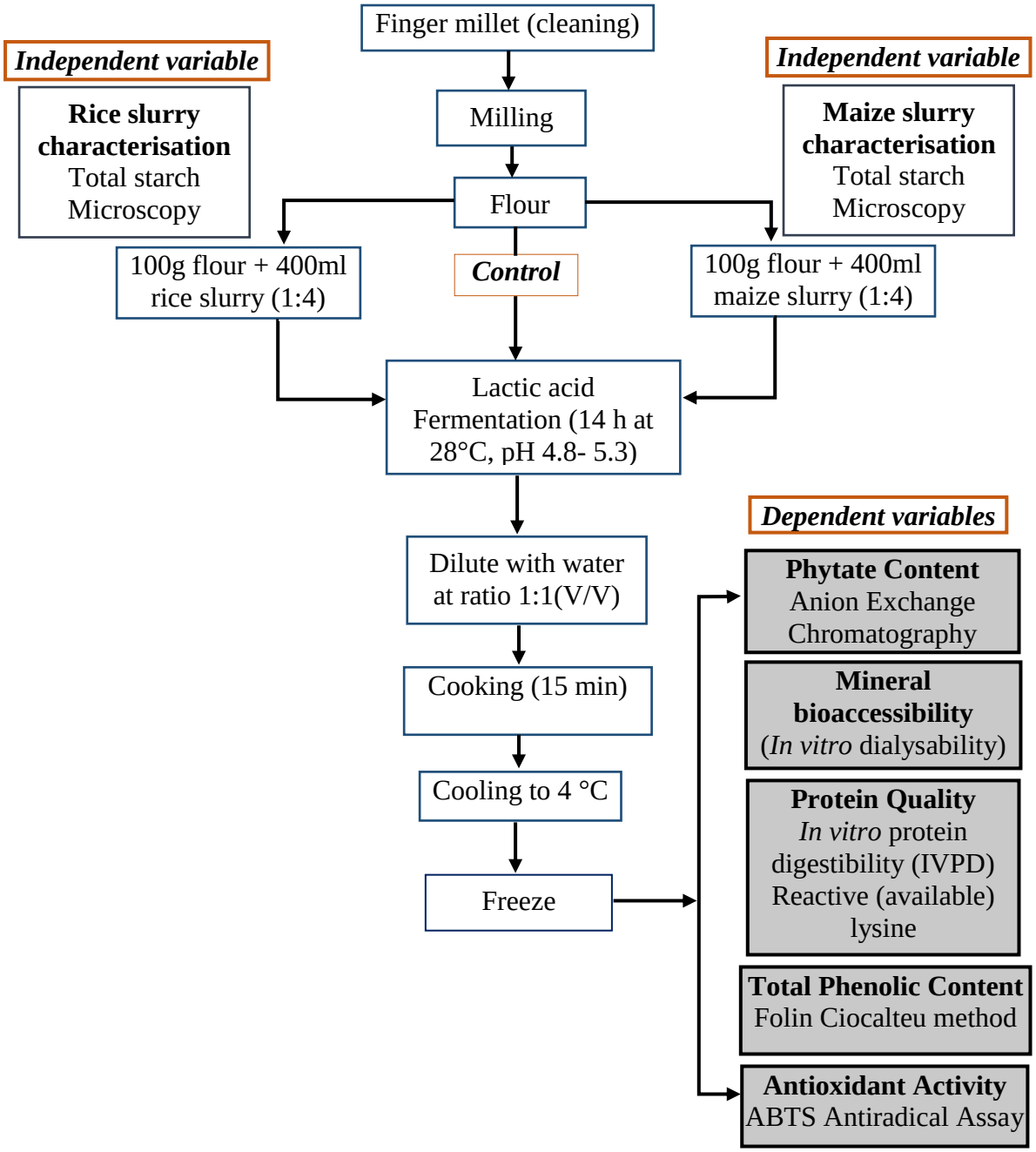


Figure 3.13. Process flow for preparation, and characterisation of three fermented finger millet-based beverages: finger millet and maize (M), finger millet and rice (R) and finger millet only (C)

3.2.2.2.2 Scanning electron microscopy (SEM)

To study the microscopic structure of raw and pre-gelatinized maize and rice flour samples, a Field Emission Gun Scanning Electron Microscope (FEG-SEM) [Model Zeiss 540 Ultra] was used. A small quantity of each dry starch sample (~1 mg) was mounted firmly on the surface of an aluminium stub with double-sided adhesive tape and sputter-coated with carbon. Stubs were fixed on the microscope and samples observed under an acceleration voltage of 1.00 kV.

3.2.2.2.3 Light and polarized microscopy

Raw maize and rice flour samples, and pre-gelatinised starch slurries were observed under light microscopy for the appearance of birefringence (polarised light mode) and presence of starch (non-polarised light mode), respectively, after staining with iodine solution using a method described by Bamidele, Duodu and Emmambux (2017). Approximately 5 mg of starch samples were suspended in 2 mL 30% glycerol solution and mixed gently by vortexing. A drop of iodine stain solution was added, and the suspension mixed. Stained slides were prepared for viewing by putting a drop of the suspension on a glass slide and covered with a glass coverslip. These were observed using a Nikkon Optiphot Transmitted Light Microscope (Tokyo, Japan) fitted with a Nikkon DXM 1200 digital camera (Tokyo, Japan).

3.2.2.2.4 Phytate content

The phytate content was determined by the indirect quantitative method described by Fruhbeck, Alonso, Marzo and Santidrián (1995). Approximately 1 g of dried sample was extracted with 20 ml of 2.4% conc. HCl (0.66 N) for 2 h on an electronic shaker followed by centrifugation at 2 819 x *g* for 30 min. From the recovered clear supernatant, 2 g was collected and diluted with distilled water at a ratio of 1:20. The pH of the dilute extract was adjusted to 6.0 using 0.5 M NaOH followed by anion-exchange purification using polypropylene solid-phase extraction cartridges with 20 µm porosity polyethylene frits (CAT No. 57176, Sigma- Aldrich) and Dowex 1 x 4 ion-exchange resin [4% cross-linkage, chloride form, 100 – 200 mesh] (CAS number 69011-19-4, P428590-100G, Sigma- Aldrich). Purified samples (3 ml) were reacted with Wade reagent for 30 min and absorbance measured at 500 nm against distilled water. Sodium phytate solutions containing dodecasodium salt hydrate from rice (CAS number 14306-25-3, P8810-25, Sigma- Aldrich) at concentrations between 5 and 50 µg/mL were used to produce a standard calibration curve.

3.2.2.2.5 Mineral bioaccessibility using *in vitro* dialysability assay

Cooked finger millet-based formulations were analysed for bioaccessibility of minerals (zinc and iron) according to the *in vitro* dialysability method of Miller, Schricker, Rasmussen and Van Campen (1981). The technique mimics human gastrointestinal digestion by using digestive enzymes, bile salts and dialysis tubing which is selectively permeable to divalent cationic minerals. The amount of minerals that passes through the tubing from the dialysate represents the amount of minerals available for human absorption. Digestive enzymes, bile salts, and dialysis tubing used in the study were pepsin (Sigma- Aldrich, P-7000), pancreatin (Sigma- Aldrich, P-1750), bile extract (Sigma- Aldrich, B-8631) and Spectra/Por 7 (Ø = 20.4 mm) tubing with a molecular weight cut-off (MWCO) of 10 kDa (G.I.C. Scientific, Johannesburg, South Africa).

Gastric stage

Approximately 6 g of finely milled formulations were measured in triplicate and mixed with 40 mL deionized water in 250 ml Erlenmeyer flasks. The pH was adjusted to 2.0 by adding 6 M HCl and the mixture was left to equilibrate for 10 min. Freshly prepared (1.8 g) pepsin solution (16 g pepsin in 100 mL of 0.1 M HCl) was added and the volume of the sample was made up to 60 mL with deionized water. Mixed samples were incubated at 37°C in a shaking water bath for 120 min. Following incubation, 12 mL aliquots were pooled from each flask for titratable acidity measurement and the rest of the digests were stored in a freezer for further analysis.

Titratable acidity

Homogenous aliquots (12 mL) of the gastric digest were cooled to 20°C after which 3 g of freshly prepared pancreatic mixture (4 g pancreatin, 25 g bile extract in 1 litre of 0.1 M $NaHCO_3$) was added. The pH of the mixture was adjusted to 7.5 using 0.5 M NaOH and left to equilibrate for 30 min. Titratable acidity was defined as the amount, in moles, of 0.5 M NaOH required to adjust the pH of the mixture to pH 7.5.

Intestinal stage

Homogenous aliquots (12 mL) of gastric digest were measured in triplicate into 250 ml Erlenmeyer flasks and placed in a water bath for 5 min at 37°C. Twenty-centimeter segments (15 cm from clamp to clamp) of the dialysis tubing containing 15 mL $NaHCO_3$ solution (equivalent in moles to NaOH used to adjust pH of digests to pH 7.5) were placed immediately

into the flasks and closed with parafilm to minimize CO_2 losses. Flasks were incubated in a shaking water bath for 30 mins at 37°C after which 3 g freshly prepared pancreatic mixture was added. These were further incubated in a shaking water bath for 120 min at 37°C. After the final incubation stage, dialysis bags were rinsed once with deionized water, and contents were quantitatively transferred into sterile 15 mL centrifuge tubes. Dialysates were acidified with 450 µL HNO_3 (65%) and centrifuged at 3000 rpm for 10 min. The supernatant was decanted into sterile 15 mL centrifuge tubes and kept frozen until analysis.

Mineral analysis

The measurement of bioaccessible (dialyzable) iron and zinc in the dialysates was performed in duplicate using Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) at Stellenbosch University's Central Analytical Facilities (CAF). To determine iron and zinc content, cooked flour samples were first digested using concentrated nitric acid plus hydrogen peroxide before analysis by ICP-AES. Bioaccessible minerals (iron and zinc) were calculated as a percentage (%) of mineral in the dialysate compared to the total mineral content in the digests while the amount of bioaccessible iron and zinc, expressed as mg/100 g, was calculated from the mineral content of formulations and % bioaccessible mineral.

3.2.2.2.6 *In vitro* protein digestibility (IVPD)

The *in vitro* protein digestibility (IVPD) was determined following the modified pepsin digestibility method described by Da Silva, Taylor and Taylor (2011). Approximately 200 mg flour was suspended in 2 ml distilled water in centrifuge tubes and immersed in a boiling water bath for 15 min. Following incubation, 5 ml, pH 2.0 of 0.1 M citrate buffer was added, and the mixture thoroughly mixed by vortexing after which 28 ml (pH 2.0) of citrate buffer mixed with pepsin (CAS number 9001-75-6, P7000-25G, Sigma- Aldrich, Pepsin from porcine gastric mucosa powder ≥250 units/mg solid) in the ratio 131 mg pepsin per 100 ml buffer solution was added. The mixture was incubated at 37°C for 2 h in a shaking water bath. After terminating the enzyme reaction with 2 M NaOH, the digests were centrifuged at 2000 x *g* for 10 minutes and the supernatant discarded. The residue was washed once with 35 ml distilled water, re-centrifuged at 2000 x *g* for 10 minutes and the residue dried overnight. The initial protein content of samples and protein content of the residue, expressed as total nitrogen (N×6·25), were determined by Dumas combustion using a Dumatherm nitrogen analyser (DT N64+, Gerhardt Konigswinter, Germany). Protein digestibility was calculated as the difference

between the total protein and residual protein after pepsin digestion expressed as a percentage of the total protein.

3.2.2.2.7 Reactive (chemically available) lysine

Determination of the quantity of nutritionally available lysine molecules was done using the modified rapid dye-binding lysine (DBL) method of Kim, Kim, Ma and Chung (2007). In this technique, the food sample is reacted with Crocein Orange G dye and the dye-binding capacity (DBC) of basic protein groups is measured which is equivalent to the sum of total histidine, arginine, and reactive lysine in the food samples. To measure the quantity of chemically reactive lysine, the food sample is treated with propionic anhydride which blocks the free ε-amino groups of reactive lysine, and the difference in DBC before and after propionation is determined. Briefly, finely milled samples were measured in duplicate (Treatment A and B) into centrifuge tubes and 5ml sodium acetate solution was added into each of the treatments. Into treatment B, 0.2 m propionic anhydride was added, and samples were mixed in a shaker at 300 rpm for 15 min. A solution of Crocein Orange G (70% dye content) [CAT number 27965, Sigma- Aldrich] in oxalic acid-acetic acid phosphate buffer (pH 1.25) was added to all samples, and the mixture was shaken vigorously to allow for electrovalent binding of dye with the basic amino acids. Aliquots of the supernatant were diluted with oxalic acid- acetic acid phosphate buffer and the amount of dye remaining in solution was measured spectrophotometrically at 482 nm. The difference in DBC between untreated (A) and samples treated (B) with propionic anhydride was equivalent in moles to the reactive lysine content of food samples.

3.2.2.2.8 Total phenolic content (TPC)

TPC was determined using the Folin-Ciocalteu (F-C) method adapted for the use of 96-well microplates according to Ainsworth and Gillespie (2007). In this assay, phenolic extracts are reacted with the yellow F-C reagent to form blue complexes whose absorbance (measured spectrophotometrically) is proportional to the phenolic content of the sample (Singleton, Orthofer & Lamuela-Raventós, 1999). Phenolic extracts were prepared by extracting 1 g of the milled sample with 10 ml acidified methanol [1% (v/v) conc. HCl in methanol] by magnetic stirring for 2 h. The mixture was centrifuged at 1509 x g for 10 min at room temperature and the supernatant collected. The residue was rinsed twice with an additional 10 ml acidified methanol, reextracted for 30 mins between each rinse, centrifuged as above and the supernatants were pooled. An aliquot (100 µl) of each extract was mixed, in triplicate, with

200 µl of 10% F-C phenol reagent (Merck, Germany) in Eppendorf tubes, vortexed and 800 µl of 250 mM sodium carbonate (Na_2CO_3) added in each tube. Distilled water and catechin hydrate (CAS number 225937-10-0, C1251-10G, Sigma-Aldrich, ≥ 98% powder) were used as reaction blank and standard, respectively. The tubes were incubated in the dark at room temperature for 2 h and the absorbance of 200 µl of the reaction mixture measured at a wavelength of 765 nm. Total phenolic content was calculated as catechin equivalent (CE) mg/g dry matter.

3.2.2.2.9 Antioxidant activity (AA)

Methanolic extracts, prepared as for TPC assay, were diluted fifteen times with acidified methanol and analysed for antioxidant activity using the 2,2´-azinobis [3 ethylbenzothiazoline-6-sulphoric acid] (ABTS) radical scavenging capacity assay as described by Apea-Bah, Minnaar, Bester and Duodu (2014). The $ABTS^+$ radical solution was prepared by mixing equal volumes of 8 mM ABTS diammonium salt (CAS number 30931-67-0, A1888-5G, Sigma-Aldrich, ≥98%) and 3 mM potassium persulphate, both prepared in distilled deionized water and reacting for 12- 16 h in the dark at room temperature. The working solution was prepared by mixing 1 ml of $ABTS^{\cdot+}$ solution with 29 ml of pH 7.4 phosphate buffer containing 150 mM sodium chloride (NaCl). Methanol extracts (10 µl) and Trolox [(±)-6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid] (CAS number 53188-07, Cat No 23,881-3, Sigma-Aldrich, 97%) standards (10 µl) were mixed with 290 µl working solution in the wells of a 96-well microplate and reacted in the dark for 30 min. The absorbance of the reaction mixture was read at 734 nm and the ABTS radical scavenging capacity calculated and expressed as micromole Trolox equivalent per gram of sample (µmol TE/g) on a dry weight basis.

3.2.2.3 Statistical analysis

To test the effect of starch source, fermentation, and cooking on dependent variables, experimental data were statistically analysed by One-Way Analysis of Variance (ANOVA) using StatSoft STATISTICA Version 8 (StatSoft, Inc., Tulsa, OK, USA). Discrimination of means by multiple comparison procedure was done using Fischer's least significant difference [LSD] test at 95% confidence level ($P < 0.05$). Experiments were replicated at least twice, and the Excel Quartile function was used to identify and eliminate outliers.

3.2.3 Results and discussion

3.2.3.1 Total starch

Table 3.5. Total starch content of maize and rice starch slurries

Sample	Total starch (% dry basis)
Maize slurry	$85.47^{b} \pm 0.48$
Rice slurry	$73.71^{a} \pm 0.89$

Mean values with different superscripts indicate significant differences ($P < 0.05$).

The total starch content of maize and rice starch slurries is presented in Table 3.5. Maize starch slurry had a significantly higher ($p < 0.05$) total starch content than the rice starch slurry. Generally, starch is the most abundant storage carbohydrate in plants (Verma, Kumar, Zaidi, Verma, Jaiswal, Singh, Singh & Agrawal, 2018), including cereal grains such as maize and rice, and is composed of two distinct glucose polymers: amylose and amylopectin (Yan, Pan, Jiang & Wu, 2009). The high starch content of cereal slurries can be due to the gelatinisation effect on starch granules when heated in excess water which disrupted the crystalline regions followed by leaching out of amylose (Sagum & Arcot, 2000) to form a viscous starch slurry. According to Singh, Kaur and Shevkani (2014) the maize endosperm, which accounts for 82-83% of the maize grain, contains approximately 85% starch which is comparable to the starch content of the maize slurry used in this study. Although other reports acknowledge that starch is the major component in the maize kernel, lower starch contents in the range of 72-73% have also been reported (Sofi, Wani, Rather & Wani, 2009; Orhun, 2013). In a study by Hu, Zhao, Duan, Linlin and Wu (2004) fifteen rice varieties were investigated for total starch and values ranging from 72.3 to 83.5% were reported. The starch content in the slurries was within reported ranges of starch content in the respective cereal grains.

3.2.3.2 Microstructure of raw and pre-gelatinised samples

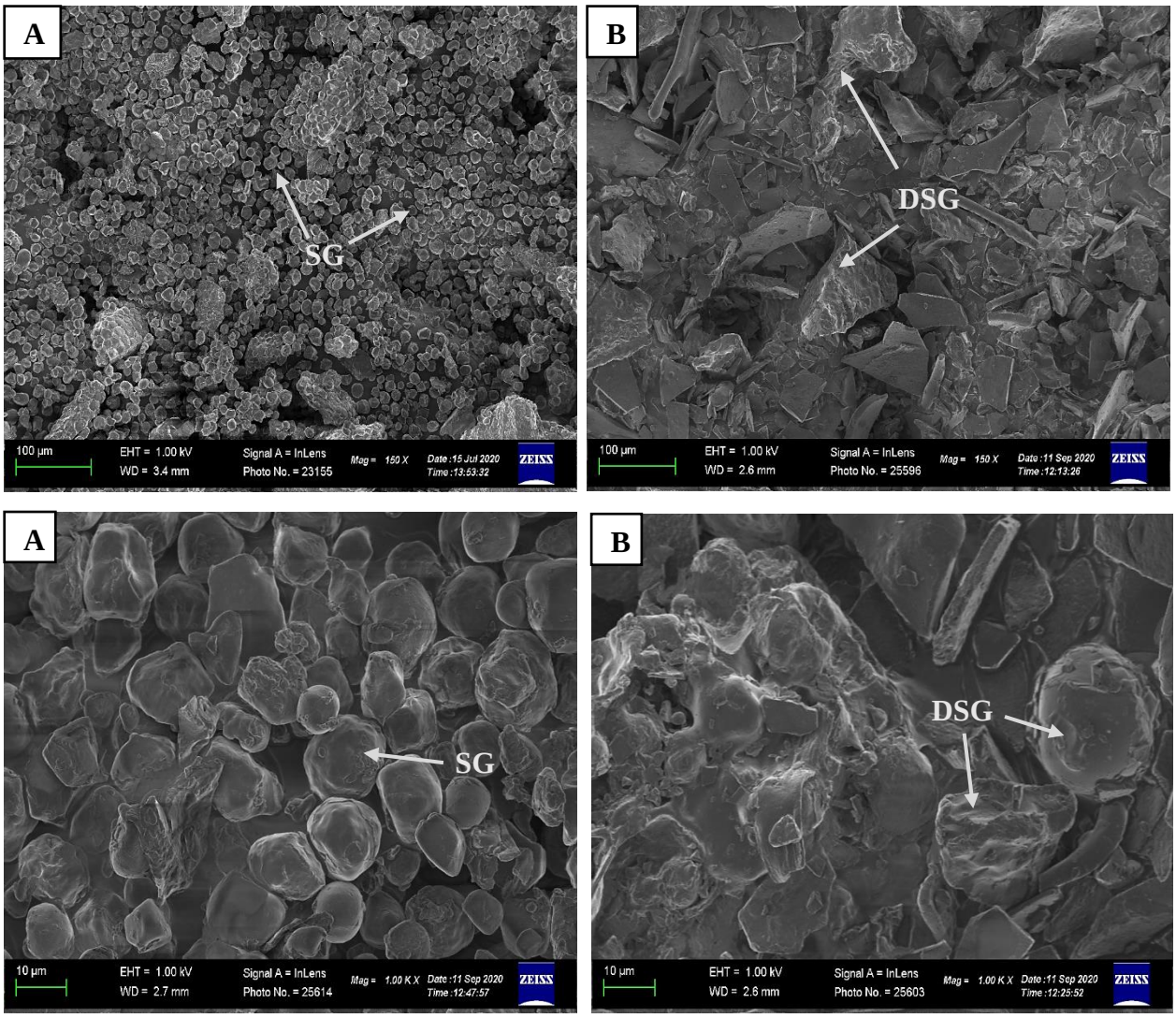


Figure 3.14. Scanning electron micrographs of milled raw maize flour (A) and pre-gelatinized maize starch slurry fractions (B). Magnification 150 x (scale bar 100 µm) and 1.00 x (scale bar 10 µm). SG- starch granule; DSG- disintegrated starch granule

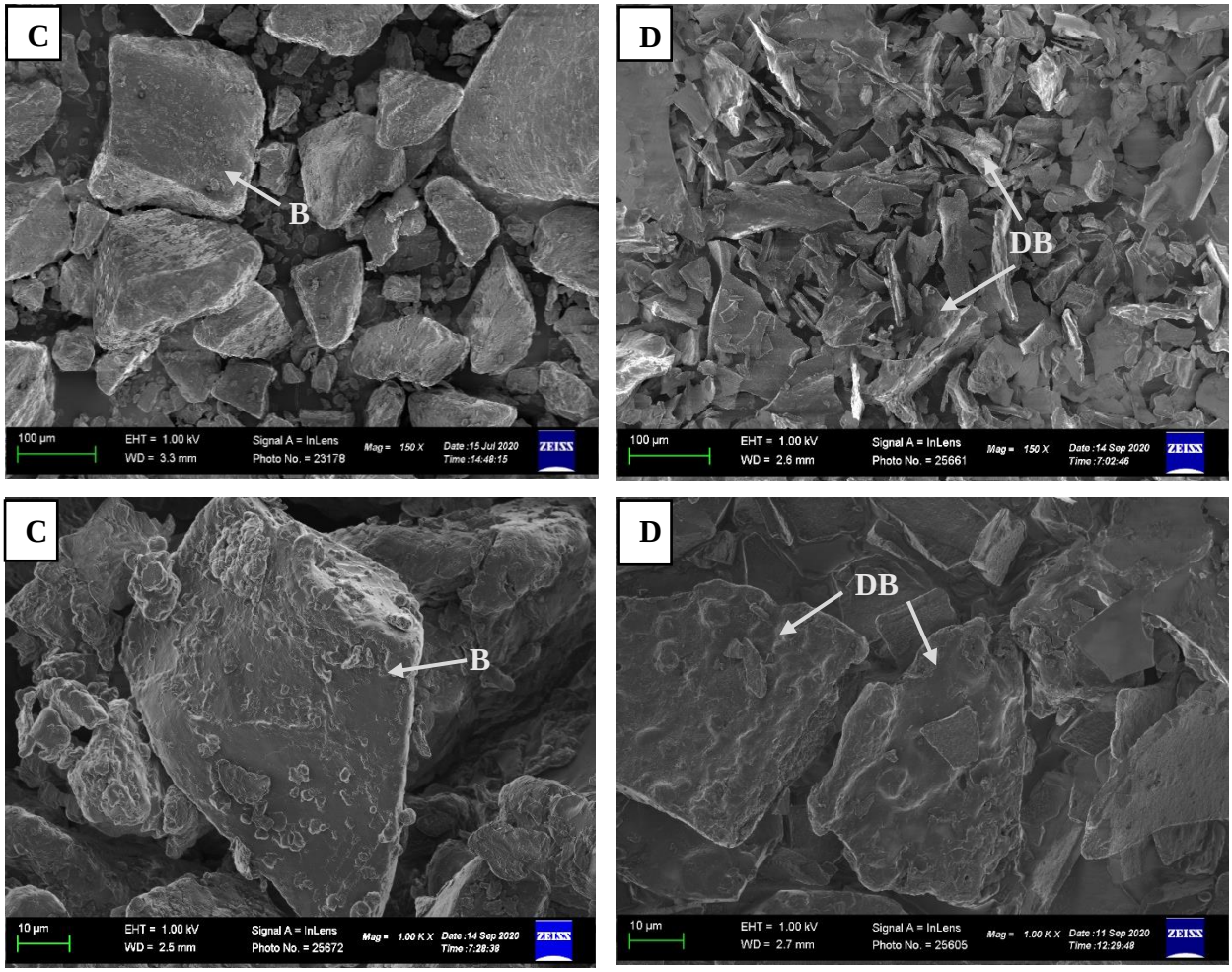


Figure 3.15. Scanning electron micrographs of milled raw rice flour (C) and pre-gelatinized rice starch slurry fractions (D). Magnification 150 x (scale bar 100 µm) and 1.00 x (scale bar 10 µm). B- block-let; DB- disintegrated block-let

The microstructure of raw maize and rice flour and pre-gelatinized starch slurries is shown in Figures 3.14 and 3.15. A comparison of the micrographs obtained before cooking (A and C) and after cooking (B and D) shows that maize starch granules lost their integrity after cooking and rice starch block let material (likely to consist of starch granules and protein bodies) was disintegrated during the cooking process. The significant variation in microstructure is an indication that maize and rice starch granules underwent gelatinization leading to the release of viscous starch polymer material hence the rough sheet-like structures observed after dehydration of slurries (Figure 3.14B and Figure 3.15D). Starch occurs in plant tissues as highly ordered microscopic granules of varying size, shape, and molecular arrangement. When heated in excess water, starch granules undergo an order-to-disorder transition process and

eventually break down into a mixture of polymers in solution, a process known as gelatinization (Ratnayake & Jackson, 2008; Kumar, Kumar, Sharma, Kaur, Chunduri, Chawla, Sharma, Singh & Garg, 2016b).

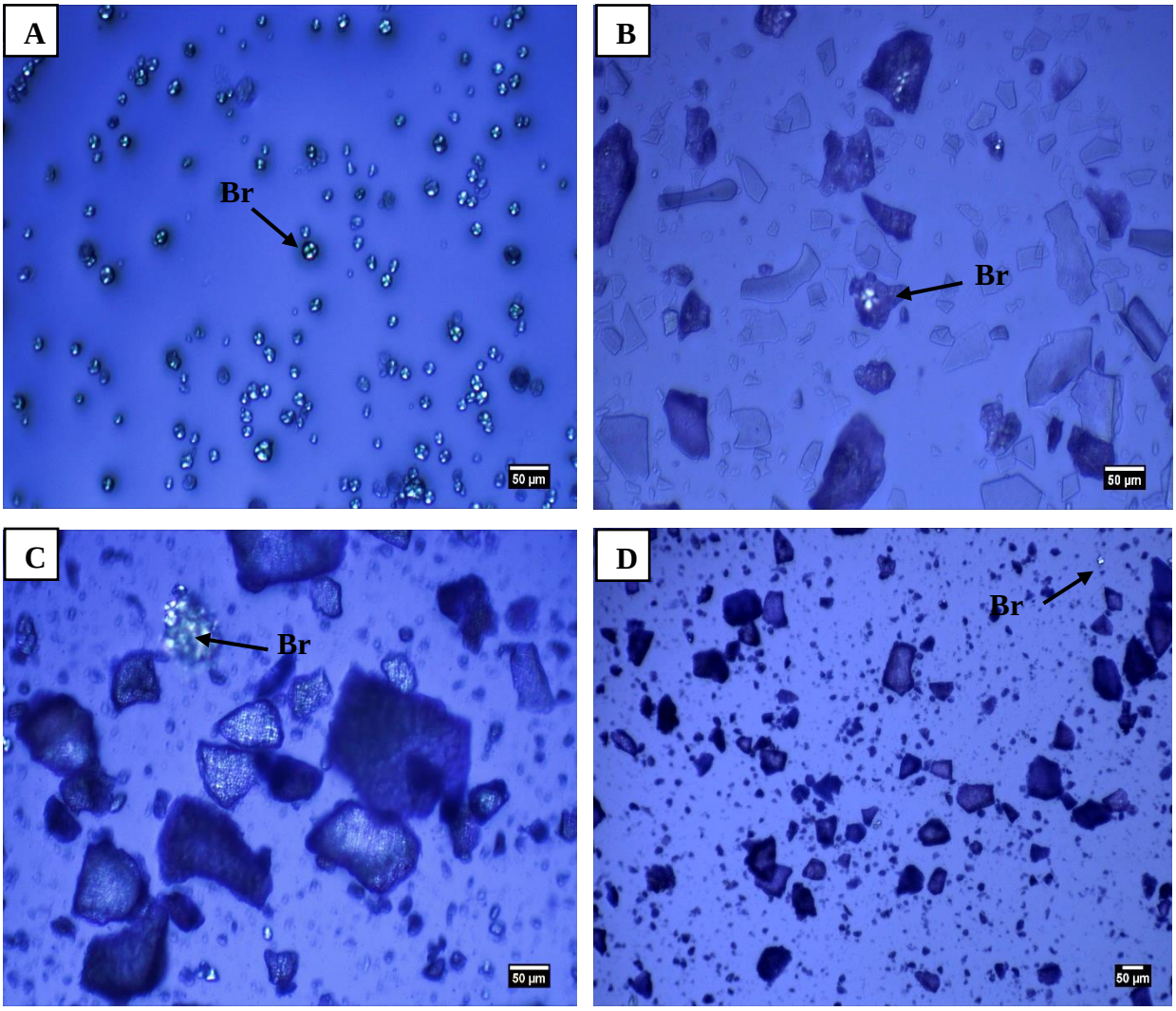


Figure 3.16. Polarized light micrographs of milled raw maize (A) and raw rice (B) flour, and pre-gelatinized maize starch (C) and rice starch (D) slurry fractions with iodine solution stain. Scale bar 50 µm. Br- birefringence

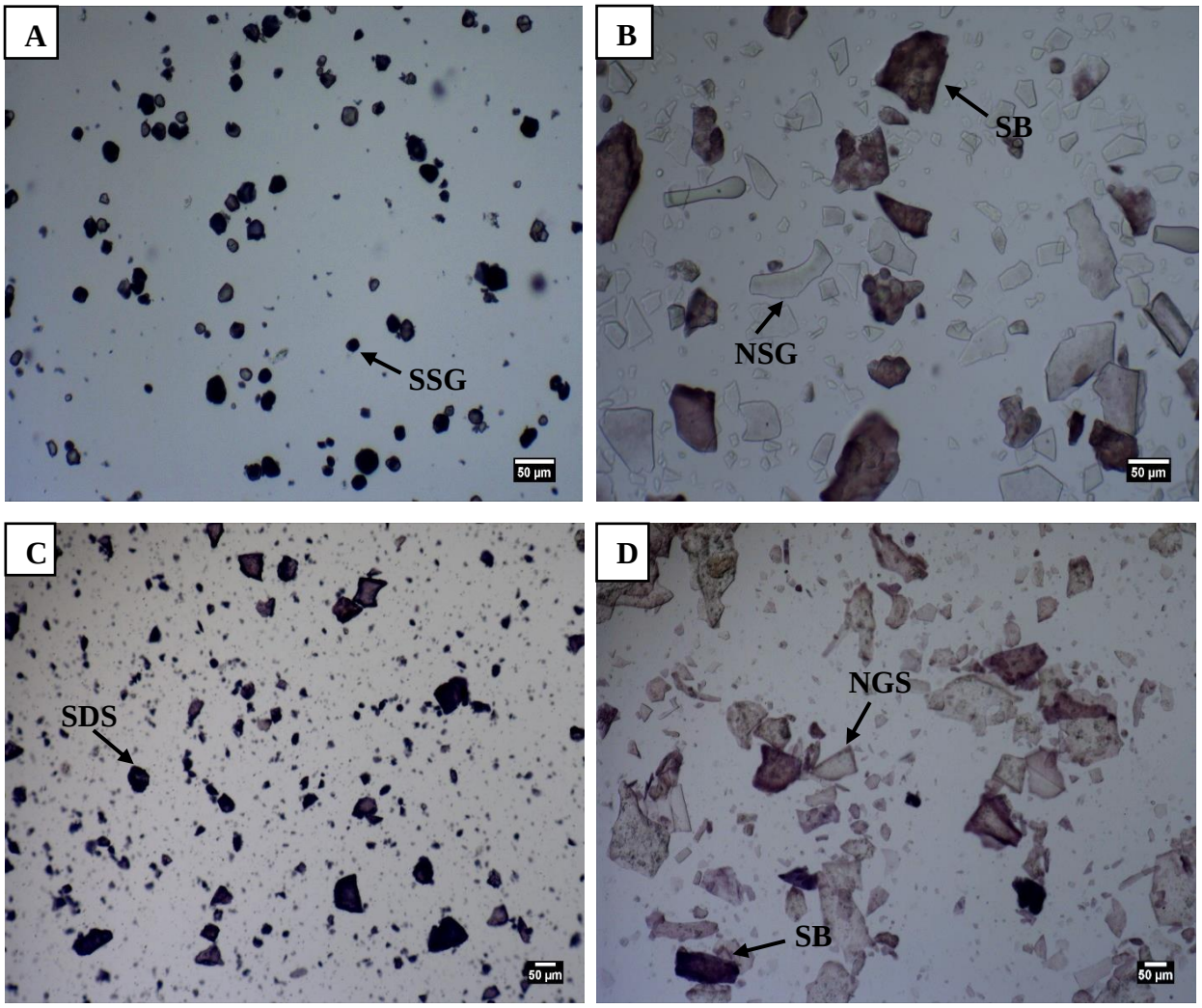


Figure 3.17. Non-polarized light micrographs of milled raw maize (A) and rice (B), and pre-gelatinized maize starch (C) and rice starch (D) slurry fractions with iodine solution stain. Scale bar 50 µm. SSG- stained starch granule; NSG- non-starch glass-like structure; SDS- stained disintegrated starch; SB- stained block-let

Light micrographs of raw maize and rice flour and their respective starch slurries show optical birefringence of raw undisrupted starch granules and block-let material, the decrease in birefringence after cooking as well as the presence of starch in slurries used for fermentation (Figure 3.16 and Figure 3.17). The presence of Maltese crosses in starch granules of the raw maize and rice flour is evidence of the semicrystalline molecular arrangement of starch granules (Pinto, Moomand, Vanier, Colussi, Villanova, Zavareze, Lim & Dias, 2015). There is, however, evidence that some starch granules for both maize and rice slurry (Figure. 3.16B and D) did not gelatinize completely as shown by traces of birefringence in slurries obtained after cooking. In their native state, starch granules exhibit an orderly crystalline region and

disorderly amorphous region. This semicrystalline arrangement gives starch granules a characteristic birefringence pattern when observed under a light microscope, which varies in intensity based on the relative crystallinity and starch granule size (Ratnayake & Jackson, 2008; Kumar *et al.*, 2016b). The amylose domain is responsible for the blue-black colour formed in the presence of iodine solution stain (Cheng & Tsai, 2016), therefore the identification of starch in cooked slurries using the iodine solution test in this study. Overall, micrographs show that both maize and rice slurries contained starch which stained blue-black and other non-starch glass-like material (Figure 3.17B and Figure 3.17D).

3.2.3.3 Phytate content

Table 3.6. Effect of starch source during fermentation on phytate content of finger millet-based beverages

Treatment	mg phytate/100 g (dry weight basis)
Finger millet and maize starch	
Unfermented and uncooked	$819.88^{bc} \pm 16.02$
Fermented and uncooked	$706.86^{a} \pm 64.48$
Unfermented and cooked	$873.31^{cde} \pm 15.26$
Fermented and cooked	$704.11^{a} \pm 11.78$
	$776.04^{A} \pm 81.80$*
Finger millet and rice starch	
Unfermented and uncooked	$867.62^{cd} \pm 10.06$
Fermented and uncooked	$821.27^{bc} \pm 54.06$
Unfermented and cooked	$877.82^{cde} \pm 33.88$
Fermented and cooked	$786.04^{b} \pm 7.40$
	$838.19^{B} \pm 47.46$*
Finger millet only	
Unfermented and uncooked	$910.60^{de} \pm 38.31$
Fermented and uncooked	$828.08^{bcd} \pm 12.90$
Unfermented and cooked	$923.21^{e} \pm 45.59$
Fermented and cooked	$830.28^{bcd} \pm 5.90$
	$873.04^{C} \pm 52.94$*

*Overall effect of starch source

Values within the same column followed by the same upper-case letters are not significantly different ($P > 0.05$).

Mean values in the same column bearing the same lower-case letters are not significantly different ($P > 0.05$).

The effect of starch source on phytate content of finger millet-based beverages is shown in Table 3.6. The starch source, as an independent variable, had a significant overall effect ($P < 0.05$) on the phytate levels of samples. Finger millet-based beverages with added maize or rice starch had relatively lower phytate content than the finger millet only samples, which may suggest that the addition of extra starch exogenously may contribute to the reduction of phytate during fermentation. The higher proportion of starch in the samples may have enhanced the activity of microbes involved in fermentation leading to better microbial enzyme activity inclusive of microbial phytases which hydrolyze phytate to lower inositol phosphates (Gibson, Bailey, Gibbs & Ferguson, 2010).

According to Harlow *et al.* (2015), there is a clear link between starch concentration and microbial activity. The use of different botanical sources of starch has an influence on microbial enzyme activity and fermentation capacity which can be attributed to physical structure and composition of starch granules (Bowman & Firkins, 1993; Harlow *et al.*, 2015). These attributes have a bearing on the availability of the substrate for fermentation, which reflects as variations in the properties of the final product between the different fermentation substrates. This can explain the significantly lower overall phytate content in finger millet samples with added maize and rice starch (776.04 mg/100 g and 838.19 mg/100 g respectively) compared to that of finger millet only samples (873.04 mg/100 g) (Table 3.6).

Table 3.7. The overall effect of fermentation and cooking on phytate content of finger millet-based beverages

Sample	**Treatment**	**mg /100 g**	**Treatment**	**mg/100 g**
Finger millet and maize starch	Unfermented	$846.60^{B} \pm 32.44$	Uncooked	$763.37^{A} \pm 74.82$
	Fermented	$705.48^{A} \pm 41.48$	Cooked	$788.71^{A} \pm 93.48$
Finger millet and rice starch	Unfermented	$872.72^{B} \pm 23.04$	Uncooked	$844.44^{AB} \pm 43.06$
	Fermented	$803.66^{A} \pm 39.54$	Cooked	$831.93^{A} \pm 54.85$
Finger millet only	Unfermented	$916.90^{B} \pm 38.29$	Uncooked	$869.34^{AB} \pm 51.93$
	Fermented	$829.18^{A} \pm 9.05$	Cooked	$876.74^{B} \pm 58.62$

For each analysis, mean values in the same row with different letters are significantly different ($P < 0.05$)

Table 3.7 shows the overall effect of fermentation and cooking on the phytate content of finger millet samples. Fermentation was effective in significantly reducing ($p < 0.05$) the phytate levels of finger millet-based samples. Similar observations were reported by different authors in finger millet (Gabaza *et al.*, 2018a) wheat bran (Zhao, Guo & Zhu, 2017) rye, wheat, and oat (Reale, Konietzny, Coppola, Sorrentino & Greiner, 2007). The apparent decrease in phytate levels after fermentation can be due to dephosphorylation of phytate by low pH activated endogenous cereal phytases (Reale *et al.*, 2007). Cereal grains exhibit phytase activity with an optimal activity interval between pH 5 and 5.5. During fermentation, lactic acid bacteria contribute to phytate degradation by providing favourable conditions for endogenous phytase activity (Svanberg, Lorri & Sandbeag, 1993; Greiner & Konietzny, 2006; Reale *et al.*, 2007). The final pH (4.89-5.17) of finger millet-based beverages was within the optimum range for cereal phytase activity.

Cooking as a processing step, for both fermented and unfermented samples, did not have a significant effect on the phytate content. Similarly, Duodu, Minnaar and Taylor (1999) and Kayodé, Linnemann, Nout and Van Boekel (2007) observed that cooking of sorghum porridge using normal household cooking procedures did not cause a significant decrease in phytate content. This effect can be partially linked to the heat destruction of phytase during heat processing which may have resulted in complete loss of phytase activity or the use of processing temperatures that are not sufficient to cause significant degradation of phytic acid (Plaami & Kumpulainen, 1995; Duodu *et al.*, 1999; Reale *et al.*, 2007). Normal cooking procedures may not destroy appreciable amounts of phytate in foodstuffs especially if the phytate occurs complexed with proteins and/or cations in natural products (De Boland, Garner & O'Dell, 1975).

3.2.3.4 Mineral bioaccessibility

Table 3.8. Effect of exogenous addition of starch on iron (Fe) and zinc (Zn) content, and their bioaccessibility in finger millet-based beverages

Treatment	Fe content (mg/100 g, dry basis)	Percentage bioaccessible iron	Amount of bioaccessible iron [mg/100 g, dry basis]	Zn content (mg/100 g, dry basis)	Percentage bioaccessible zinc	Amount of bioaccessible zinc [mg/100 g, dry basis]
			Finger millet and maize starch			
Unfermented and cooked	$18.14^{ab} \pm 0.36$	$3.89^{c} \pm 0.35$	$0.69^{c} \pm 0.06$	$2.26^{d} \pm 0.02$	$44.79^{c} \pm 3.77$	$1.01^{c} \pm 0.09$
Fermented and cooked	$16.54^{a} \pm 3.55$	$3.39^{b} \pm 0.75$	$0.56^{b} \pm 0.12$	$2.03^{a} \pm 0.02$	$51.15^{d} \pm 4.30$	$1.00^{c} \pm 0.09$
	$17.07^{A} \pm 2.88$*	$3.64^{B} \pm 0.63$*	$0.63^{B} \pm 0.12$*	$2.10^{A} \pm 0.12$*	$47.97^{B} \pm 5.11$*	$1.01^{B} \pm 0.08$*
			Finger millet only			
Unfermented and cooked	$18.81^{ab} \pm 0.04$	$2.11^{a} \pm 0.32$	$0.40^{a} \pm 0.06$	$2.10^{b} \pm 0.00$	$18.76^{a} \pm 1.81$	$0.38^{a} \pm 0.04$
Fermented and cooked	$20.40^{b} \pm 0.22$	$1.97^{a} \pm 0.09$	$0.40^{a} \pm 0.02$	$2.17^{c} \pm 0.03$	$25.48^{b} \pm 2.49$	$0.52^{b} \pm 0.05$
	$19.87^{A} \pm 0.84$*	$2.04^{A} \pm 0.24$*	$0.40^{A} \pm 0.04$*	$2.14^{B} \pm 0.04$*	$22.12^{A} \pm 4.04$*	$0.45^{A} \pm 0.08$*

*Overall effect of starch source
Values within the same column followed by the same upper-case letters are not significantly different (P > 0.05).
Mean values in the same column bearing the same lower-case letters are not significantly different (P > 0.05).

Table 3.8 presents the influence of exogenous addition of starch and fermentation on iron (Fe) and zinc (Zn) content and their bioaccessibility in finger millet-based beverages. Of the two starch sources (rice and maize) only the finger millet-based beverages with added maize starch were studied due to analytical constraints. The addition of starch (maize) slurry did not have a significant effect ($p > 0.05$) on the overall Fe content of finger millet-based beverages, however, reduced slightly the Zn content of samples. Interestingly, beverages with added maize starch had higher overall bioaccessible Fe (0.63 mg/100 g) and Zn (1.01 mg/100 g) and higher overall percentage bioaccessible Fe (3.64%) and Zn (47.97%) compared to finger millet only samples (0.40 and 0.45 mg/ 100 g, and 2.04 and 22.12%, respectively). This may suggest a complexation reaction between mineral ions and hydroxyl groups of added starch. The subsequent release of mineral ions during digestion may have improved mineral bioaccessibility *in vitro*.

Previous studies have demonstrated that starch (granular, native, or pregelatinized form) functional groups, mainly hydroxyl (OH) groups, can act as important coordination sites for cations such as Fe and Zn (Łabanowska, Kurdziel, Bidzińska, Fortuna, Pietrzyk, Przetaczek-Rożnowska & Rożnowski, 2013; Luo, Zou, Chen, Cheng, Fu & Xiao, 2016; Liu, Li, Shang & Xie, 2019; Sigdel, 2019). Cations can form complexes with glucose units of starch or starch fractions: amylose and amylopectin, via negatively charged oxygen atoms of hydroxyl groups and these complexes are held together by electrostatic forces (Łabanowska *et al.*, 2013; Sigdel, 2019). The mineral ions can, however, be released for absorption after digestion (Sigdel, 2019), which may have improved bioaccessibility in this case.

Fermentation had no significant effect ($p > 0.05$) on the Fe content of both finger millet and maize, and finger millet only samples, however, reduced slightly the Zn content of finger millet only samples (Table 3.8). Also, fermented samples with added maize slurry had a significantly lower ($p < 0.05$) percentage and amount of bioaccessible Fe compared to unfermented samples. This may suggest possible chelation of Fe by organic compounds, other than phytate, in the fermentation medium such as phenolic compounds (Baye, Mouquet-Rivier, Icard-Vernière, Picq & Guyot, 2014; Humer & Schedle, 2016; Shumoy, Lauwens, Gabaza, Vandevelde, Vanhaecke & Raes, 2017), which were significantly increased in fermented finger millet beverages with added starch (Table 3.12). A different trend was observed for bioaccessible Zn, where instead, fermented finger millet and maize, and finger millet only samples had relatively higher ($p < 0.05$) percentage bioaccessible zinc compared to unfermented beverage samples

(Table 3.8). The extent of phytate degradation during fermentation (Table 3.7) may not have resulted in observable improvement in iron bioaccessibility, but was sufficient to improve the bioaccessibility of zinc.

Table 3.9. Effect of exogenous addition of starch and fermentation on the phytate: iron and phytate: zinc molar ratios in finger millet-based beverages

	Phytate: mineral molar ratios	
Treatment	**Phytate: iron**	**Phytate: zinc**
Finger millet and maize starch		
Unfermented and cooked	4.1:1	38.3:1
Fermented and cooked	3.6:1	34.4:1
Finger millet only		
Unfermented and cooked	4.2:1	43.6:1
Fermented and cooked	3.4:1	37.9:1

Phytate: mineral ratios were calculated based on the mean phytate and mineral contents, and their molecular mass.

Phytate: mineral ratios are an important quantitative relation between phytate and mineral concentrations in food that can be used to predict the proportion of dietary minerals available for absorption (Gibson *et al.*, 2010; Van der Merwe, 2017). To achieve desirable levels of mineral absorption, it is proposed that the phytate: Fe and phytate: Zn ratios should be less than 1:1 and 18:1 respectively (Gibson *et al.*, 2010). Phytate: mineral ratios above these suggested thresholds may hinder adequate mineral absorption from the diet. In this study, the phytate: Fe ratios ranged between 3.4:1 and 4.2:1 while the phytate: Zn ratios ranged between 34.4:1 and 43.6:1. These phytate: mineral molar ratios indicate inadequate Fe and Zn bioavailability from finger millet-based beverages under study.

While fermented finger millet and maize beverage samples had a slightly lower phytate: Fe molar ratio (3.6:1) than unfermented counterparts (4.1:1) (Table 3.9), no improvements were observed in the percentage and amount of bioaccessible Fe but rather, significant losses were recorded (Table 3.8). As expected, the lower phytate: zinc ratios of fermented samples (finger millet and maize, and finger millet only), compared to unfermented samples (Table 3.9), were in correspondence with the higher bioaccessible zinc observed (Table 3.8). The negative effects

of phytate on Fe can be eliminated only if phytate levels are reduced to below 100 mg/ 100 g, dry basis (Greffeuille, Kayodé, Icard-Vernière, Gnimadi, Rochette & Mouquet-Rivier, 2011), better still if the phytate: iron molar ratio is < 0.4:1 for cereal-based diets with no iron enhancers (Hurrell, 2004; Hurrell & Egli, 2010). The increase in bioaccessible Zn after fermentation, despite molar ratios above the desired critical limit (< 18) shows a somewhat lack of correlation between phytate: mineral molar ratios and mineral bioaccessibility results. This may suggest that phytate: mineral molar ratios should not be applied alone as predictors of bioavailable minerals. Other nutrient inhibitors such as phenolic compounds and dietary fiber and enhancers should also be considered (Van der Merwe, 2017).

3.2.3.4 *In vitro* protein digestibility and reactive lysine

Table 3.10. Effect of starch source during fermentation, and cooking on protein content, *in vitro* protein digestibility and reactive (available) lysine of finger millet-based beverages

Treatment	Total Protein [% dry basis]	% *In vitro* protein digestibility	Reactive lysine [mg/g dry matter]
Finger millet and maize starch			
Unfermented and uncooked	$7.97^{c} \pm 0.40$	$38.53^{c} \pm 0.45$	$2.22^{cd} \pm 0.14$
Fermented and uncooked	$6.53^{a} \pm 0.09$	$70.60^{e} \pm 2.25$	$2.08^{bc} \pm 0.17$
Unfermented and cooked	$7.15^{ab} \pm 0.09$	$26.78^{a} \pm 1.68$	$1.99^{ab} \pm 0.04$
Fermented and cooked	$7.42^{b} \pm 0.24$	$41.46^{cd} \pm 1.32$	$1.92^{a} \pm 0.06$
	$7.32^{A} \pm 0.50$*	$47.20^{A} \pm 16.17$*	$2.05^{A} \pm 0.15$*
Finger millet and rice starch			
Unfermented and uncooked	$8.01^{c} \pm 1.16$	$38.89^{c} \pm 2.70$	$2.47^{ef} \pm 0.03$
Fermented and uncooked	$6.94^{ab} \pm 0.03$	$67.93^{e} \pm 3.67$	$2.25^{de} \pm 0.13$
Unfermented and cooked	$7.09^{ab} \pm 0.10$	$26.08^{a} \pm 1.94$	$1.93^{a} \pm 0.10$
Fermented and cooked	$7.90^{c} \pm 0.11$	$42.71^{d} \pm 1.86$	$1.96^{ab} \pm 0.05$
	$7.62^{AB} \pm 0.61$*	$45.38^{A} \pm 14.30$*	$2.15^{A} \pm 0.24$*
Finger millet only			
Unfermented and uncooked	$7.95^{c} \pm 0.21$	$69.92^{e} \pm 0.80$	$2.28^{de} \pm 0.02$
Fermented and uncooked	$7.20^{ab} \pm 0.13$	$31.96^{b} \pm 2.00$	$2.40^{ef} \pm 0.05$
Unfermented and cooked	$6.94^{ab} \pm 0.26$	$25.83^{a} \pm 3.88$	$2.16^{cd} \pm 0.04$
Fermented and cooked	$8.07^{c} \pm 0.24$	$41.26^{cd} \pm 2.09$	$1.98^{ab} \pm 0.03$
	$7.72^{B} \pm 0.52$*	$43.40^{A} \pm 17.15$*	$2.20^{A} \pm 0.17$*

*Overall effect of starch source

Values within the same column followed by the same upper-case letters are not significantly different ($P > 0.05$).

Mean values in the same column bearing the same lower-case letters are not significantly different ($P > 0.05$).

Table 3.10 shows the effects of starch source during fermentation as well as the effects of cooking on the protein content, percentage *in vitro* protein digestibility (IVPD) and reactive (available) lysine of finger millet-based beverages. The total protein content varied across the samples from 6.53% to 8.07%. Essentially, fermented and uncooked, and unfermented and cooked finger millet-based beverages showed a slightly lower but significant ($P < 0.05$) protein content than the rest of the samples. Adeyanju *et al.* (2019) reported a similar reduction in the protein content of sorghum-amaranth composite flour after fermentation without cooking as well as lower protein content in fermented and cooked sorghum-amaranth beverages compared to cooked and fermented counterparts. The decrease in protein content after fermentation can be associated with microbial hydrolysis and metabolism of amino acids to ammonia and other volatile ~~flavor~~ compounds (Pranoto *et al.*, 2013). Interestingly, unfermented and cooked samples had lower protein content than unfermented and uncooked samples. The reason for this is not clear but it may suggest that cooking brought about slight volatilisation of low molecular weight nitrogen compounds which could cause loss of nitrogen from the food system, therefore the slight reduction in protein content of the unfermented and cooked samples.

Overall, the addition of maize and rice starch (that is, starch source as an independent variable) did not have a significant effect ($p > 0.05$) on IVPD of the fermented and cooked finger millet-based beverages (Table 3.10). Cooking, for both unfermented and fermented samples, led to a significant decrease ($p < 0.05$) in IVPD, a well-known effect that has been reported in pearl and proso millet (Bora, Ragaee & Marcone, 2019), and other cereals such as sorghum (Duodu, Tang, Grant, Wellner, Belton & Taylor, 2001; Duodu, Nunes, Delgadillo, Parker, Mills, Belton & Taylor, 2002; Adeyanju *et al.*, 2019). The reduction in protein digestibility of cooked finger millet-based beverages can be attributed to the crosslinking of protein side chains either by disulphide crosslinking or other non-disulphide interactions between polypeptides to form indigestible protein aggregates or the possible interaction between proteins and other non-protein components such as phytate and dietary fibre to form complexes, which are less susceptible to proteolytic enzymes (Duodu *et al.*, 2002; Duodu, Taylor, Belton & Hamaker, 2003; Gulati, Li, Holding, Santra, Zhang & Rose, 2017; Joye, 2019).

Fermentation improved the IVPD of uncooked and cooked samples except for uncooked finger millet only samples. Most importantly, all fermented and cooked samples had higher IVPD

than unfermented and cooked samples. This indicates that fermentation seemed to somewhat alleviate the negative effects of cooking on IVPD. This positive effect of fermentation can be attributed to the pre-digestion of grain storage proteins by fermenting microflora and endogenous proteases into peptides and amino acids which are more soluble and accessible to digestive enzymes (Chavan *et al.*, 1988; Day & Morawicki, 2018). Another possible mechanism supported by various authors is the reduction in antinutritional factors such as phytate, which can form insoluble complexes with proteins. The reduction in phytate can be linked to the activation of cereal endogenous enzymes, particularly phytases which are activated at low pH during fermentation. The dissociation of the insoluble phytate: protein complexes improves the digestibility of proteins (Nout & Motarjemi, 1997; El Hag *et al.*, 2002; Blandino *et al.*, 2003; Nkhata *et al.*, 2018).

The starch source, whether maize or rice, did not have a significant effect ($p > 0.05$) on the reactive lysine content of finger millet-based beverages. Similarly, the reactive lysine content was not significantly altered ($p > 0.05$) by fermentation for both cooked and uncooked samples (Table 3.10). The observation in this study on the effect of fermentation on reactive lysine content of beverage samples, is similar to that of Adeyanju *et al.* (2019). Nche, Nout and Rombouts (1995), however, reported a significant increase in reactive lysine content of maize and maize-cowpea mixtures fermented for 4 days. Presumably, the short fermentation period of 14 h in this study compared to 4 days in the Nche *et al.* (1995) study, may have led to the observed no effect of fermentation on reactive lysine content in this study.

Cooking reduced the reactive lysine content of both fermented and unfermented beverages (Table 3.10). The significant reduction in lysine content following heat treatment can be due to the interaction of ε-amino groups of lysine with carbonyl groups of reducing sugars to form Maillard browning conjugates or crosslinking of lysine with other amino acids to form high molecular weight aggregates which are less chemically available (Nche *et al.*, 1995; Onyango, Noetzold, Ziems, Hofmann, Bley & Henle, 2005; De Oliveira, Coimbra, de Oliveira, Zuñiga & Rojas, 2016). The substantial decrease in reactive lysine content after cooking is consistent with reports by AwadElkareem and Taylor (2011) for *kisra*, a fermented pancake-like sorghum flatbread and Adeyanju *et al.* (2019) in a study on soured sorghum and amaranth non-alcoholic beverages. This effect was associated with the blockage of lysine molecules by Maillard-type reactions, which reduce the chemical availability of lysine.

3.2.3.5 Total phenolic content (TPC) and antioxidant activity (AA)

Table 3.11. Effect of starch source and fermentation on total phenolic content and antioxidant activity of finger millet-based beverages

Treatment	Total phenols (mg CE/g dry matter)	Antioxidant activity (µmol TE/g dry matter)
Finger millet and maize starch		
Unfermented and uncooked	$7.72^{abc} \pm 1.32$	$92.17^{bc} \pm 4.97$
Fermented and uncooked	$9.29^{d} \pm 0.71$	$110.43^{ef} \pm 1.90$
Unfermented and cooked	$7.14^{a} \pm 0.90$	$80.05^{a} \pm 5.41$
Fermented and cooked	$9.22^{d} \pm 1.50$	$96.72^{cd} \pm 4.62$
	$7.93^{A} \pm 1.42$*	$95.84^{A} \pm 10.82$*
Finger millet and rice starch		
Unfermented and uncooked	$8.45^{bcd} \pm 0.97$	$108.13^{e} \pm 5.76$
Fermented and uncooked	$9.10^{d} \pm 0.67$	$117.31^{g} \pm 6.79$
Unfermented and cooked	$7.50^{ab} \pm 0.88$	$84.27^{a} \pm 2.44$
Fermented and cooked	$8.35^{bcd} \pm 0.41$	$98.23^{d} \pm 5.67$
	$8.17^{A} \pm 0.97$*	$101.72^{AB} \pm 12.44$*
Finger millet only		
Unfermented and uncooked	$9.33^{d} \pm 0.46$	$115.57^{fg} \pm 4.00$
Fermented and uncooked	$7.77^{bc} \pm 0.96$	$112.00^{efg} \pm 3.26$
Unfermented and cooked	$9.28^{d} \pm 0.89$	$110.71^{ef} \pm 1.08$
Fermented and cooked	$8.07^{bc} \pm 1.20$	$92.03^{b} \pm 3.56$
	$8.27^{A} \pm 1.15$*	$104.64^{B} \pm 10.91$*

*Overall effect of starch source

Values within the same column followed by the same upper-case letters are not significantly different ($P > 0.05$).

Mean values in the same column bearing the same lower-case letters are not significantly different ($P > 0.05$).

Table 3.11 shows the effects of starch source and fermentation on the TPC and AA of finger millet-based beverages. Generally, the TPC and AA of the samples varied from 7.14 to 9.33 CE/g and 80.05 to 117.31 µmol TE/g, respectively (Table 3). Overall, the starch source (whether maize or rice) had no effect on TPC and AA.

Fermentation (uncooked and cooked samples) increased TPC and AA of the finger millet with added starch samples but not the finger millet only samples where fermentation decreased TPC and AA (Tables 3.11 and 3.12). Starch is a highly fermentable substrate and universal carbon source for the growth of fermenting organisms (Liu *et al.*, 2015). The addition of starch to finger millet-based beverages may have exerted a beneficial effect on fermenting microbes ultimately improving the efficiency of fermentation and mobilisation of hydrolytic enzymes such as esterases. Esterases can split ester bond between phenolic compounds and cell wall matrixes leading to the release of soluble phenolics and enhanced extractability of bound phenolic compounds (Salar, Certik & Brezova, 2012; Duodu, 2014; Adebo & Gabriela Medina-Meza, 2020), hence the increase in TPC and AA as observed.

Table 3.12. The overall effect of fermentation on total phenolic content and antioxidant activity of finger millet-based beverages

Sample	**Treatment**	**TPC**	**AA**
Finger millet and maize starch	Unfermented	$7.40^{A} \pm 1.13$	$86.11^{A} \pm 8.05$
	Fermented	$9.24^{B} \pm 1.21$	$101.56^{B} \pm 7.75$
Finger millet and rice starch	Unfermented	$7.98^{A} \pm 1.03$	$96.20^{A} \pm 13.25$
	Fermented	$8.61^{B} \pm 0.62$	$104.96^{A} \pm 11.08$
Finger millet only	Unfermented	$9.30^{B} \pm 0.67$	$113.14^{B} \pm 3.78$
	Fermented	$7.88^{A} \pm 1.05$	$98.27^{A} \pm 10.13$

For each analysis, mean values in the same row with different letters are significantly different ($P < 0.05$)

Cooking (unfermented and fermented samples) did not affect TPC of the finger millet-based beverages, which may suggest the protection of phenolic compounds during thermal processing by other food components (Duodu, 2014), but brought about a significant decrease in AA (Tables 3.12 and 3.13) as similarly observed by Dlamini, Taylor and Rooney (2007). The decrease in AA after cooking, a trend not observed with the TPC could indicate that other non-

phenolic bioactive compounds contribute to the AA of fermented finger millet-based beverages. These may include bioactive peptides released from proteins by microbial and endogenous proteolytic enzymes during fermentation (Martinez-Villaluenga, Peñas & Frias, 2017; Adebo & Gabriela Medina-Meza, 2020). The thermal degradation of such antioxidative compounds may have contributed to the reduced AA of the cooked finger millet-based samples.

Table 3.13. Overall effect of cooking on total phenolic content and antioxidant activity of finger millet-based beverages

Sample	Treatment	TPC	AA
Finger millet and maize starch	Uncooked	$8.13^{A} \pm 1.37$	$102.13^{B} \pm 10.13$
	Cooked	$7.78^{A} \pm 1.46$	$91.51^{A} \pm 9.26$
Finger millet and rice starch	Uncooked	$8.60^{B} \pm 0.94$	$113.14^{B} \pm 7.68$
	Cooked	$7.80^{A} \pm 0.85$	$93.87^{A} \pm 8.23$
Finger millet only	Uncooked	$8.13^{A} \pm 1.09$	$113.95^{B} \pm 3.97$
	Cooked	$8.47^{A} \pm 1.23$	$98.62^{A} \pm 9.64$

For each analysis, mean values in the same row with different letters are significantly different ($P < 0.05$)

3.2.4 Conclusion

The addition of an exogenous starch source during fermentation of finger millet-based beverages decreased the phytate content of beverages and increased the overall amount and percentage bioaccessibility of iron and zinc compared to finger millet only beverages. Fermentation was effective in reducing the phytate content of finger millet-based beverages (with or without added starch), whilst increasing percentage bioaccessible zinc. This was not the case with iron where fermented finger millet-based beverages had a lower amount and percentage bioaccessible iron compared to unfermented beverages. *In vitro* protein digestibility, reactive lysine content, total phenolic content and antioxidant activity were not altered by starch source, whether maize or rice. Although cooking resulted in a decrease *in vitro* protein digestibility and reactive lysine content of both fermented and unfermented finger millet beverages, fermented and cooked beverages had higher digestible protein than unfermented and cooked samples. This shows that fermentation as a processing step helped alleviate the negative effects of cooking on *in vitro* protein digestibility. Even so, fermentation

increased the total phenolic content and antioxidant activity of beverages with added starch. The findings indicate that fermentation, with or without added starch, is a cost-effective approach that can be used to improve zinc bioaccessibility, protein quality as measured by *in vitro* protein digestibility, and health-promoting properties of finger millet-based beverages.

CHAPTER 4: GENERAL DISCUSSION

4.1 Experimental approach

4.1.1 Sample preparation

The beverages studied in this research were prepared based on the concept of the traditional Indian beverage *ambali*, which is prepared from the lactic acid fermentation of a finger millet and rice composite (Antony & Chandra, 1997). This is principally a finger millet-based fermented beverage in which an exogenous source of starch is added in the form of rice. The idea was then used to prepare a similar fermented beverage that would be suitable for the sub-Saharan African region. Finger millet is already well known as an important cereal food crop in sub-Saharan Africa which is widely cultivated for food due to its ability to thrive under low rainfall with limited agriculture input requirements (Shobana *et al.*, 2013; Kumar *et al.*, 2016a). For the sub-Saharan African *ambali* equivalent, maize was selected to be used to provide the exogenous source of starch (compared to rice in the Indian *ambali*). The selection of maize as an alternative to rice was motivated by its importance as a staple food cereal in the sub-Saharan African region (FAOSTAT, 2016; Ekpa, Palacios-Rojas, Kruseman, Fogliano & Linnemann, 2019; Greyling & Pardey, 2019).

Commercially available rice and maize samp (maize grits) were used for the preparation of starch slurries as these products are readily available. The actual process of preparation and extraction of starch slurries was simple and can be easily replicated at household level. Rice and maize samp were separately boiled on a stove (to pre-gelatinise starch) following cooking instructions from the manufacturer. The cooked rice and samp were drained on a fine-mesh sieve and the leftover starch slurry was collected for use in fermentation.

The spontaneous fermentation process was used since in African communities, traditional fermented cereal-based beverages are predominantly prepared using spontaneous fermentation (Phiri *et al.*, 2019). Also, products prepared by spontaneous fermentation have the combined benefit of mixed cultures in terms of output functions such as aroma, flavour, texture and nutritional properties as opposed to pure culture or inoculated fermentations where products tend to exhibit limited features and characteristics depending on the selected strains or cultures. However, due to the low predictability of the spontaneous fermentation process, for example slow fermentation and variability of product quality, it may be unsuitable for large-scale production where knowledge of physiological and metabolic properties of microorganisms is

required (Navarrete-Bolaños, 2012). The recipe and methodology used to prepare the beverages was the lab-scale standardised methodology of the traditional *ambali* recipe as described by Alavi, Mazumdar and Taylor (2019). The inclusion of an exogenous starch source in the form of starch slurries improves the starch component of the beverages and this is of significance given that starch is an important carbon source for fermentation microflora. The use of two different starch sources (rice and maize starch slurry) during the fermentation process, was therefore studied with the view that the starches will influence the microbial properties of finger millet-based slurries which will drive differences in the nutritional and health properties of the final products.

4.1.2 Microbial identification by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS)

Following preliminary phenotypic identification methods, which included Gram reaction, catalase test, and sugar fermentation test, identification of presumable lactic acid bacteria isolates from fermented cereal-based slurries was done using protein profiling by MALDI-TOF MS fingerprinting. This is a soft ionization technique in which matrix co-crystallized samples are laser radiated to generate almost exclusively single charged ions followed by desorption and ionization steps. A time of flight (TOF) mass analyser measures the time required for the ions of different masses to migrate from the ion source to the detector. The identification of microbes is then achieved by searching databases and comparing mass spectra peptide and protein profiles of analysed samples with the reference spectra of microorganisms of interest. Scoring algorithms are used to match these profiles (Lewis, Wei & Siuzdak, 2006; Jang & Kim, 2018).

Miguel, de Castro Reis, Efraim, Santos, Lima and Schwan (2017) described the protein profile by MALDI-TOF as a rapid, reliable, and low-cost tool that can be used to identify microbial isolates. Despite its low resolving power, MALDI-TOF MS is suitable for the identification and differentiation of microbes at species level, hence, it is rapidly replacing conventional biochemical and phenotypic techniques (Jang & Kim, 2018). The low resolving power of MALDI-MS instruments, however, raises concerns over the reproducibility of profiles, hence the need to optimise sample preparation protocols and to continuously improve reference databases (Jang & Kim, 2018). Also, gene sequencing techniques such as 16S rRNA, as was done in this research, can be performed for the confirmation of identified isolates where reproducibility is questionable (Lau, Teng, Ho & Woo, 2015).

Since matrix selection is critical in ensuring good resolution during MS analysis (Lewis *et al.*, 2006), α-cyano-4-hydroxycinnamic acid (CHCA) was used in this study as it is a hard matrix and results in small homogenous crystals. Unlike a soft matrix, a hard matrix absorbs substantial amounts of laser light energy, which means that large amounts of internal energy are transferred to the analyte during desorption and ionization resulting in significant fragmentation of ions (Jang & Kim, 2018). This improves the yield of ions and allows for measurement of the analyte with high accuracy and sub-picomole sensitivity which is useful for the accurate identification and characterization of microbial proteins (Lewis *et al.*, 2006).

4.1.3 Microbial characterisation by 16s rRNA amplicon sequencing

Confirmation of bacterial species composition of finger millet-based slurries was determined by ribosomal gene sequencing based on 16s ribosomal RNA [rRNA] subunit. From an early stage of molecular taxonomic studies, ribosomal RNA genes have been used as standard phylogenetic markers in microbial identification and classification due to their ubiquitous distribution and conservation across lineages (Kim & Chun, 2014). Relative to other sequencing and identification technologies, 16S rRNA gene sequencing is technically simple, cost-effective, universal, reproducible, scalable, and is a robust criterion hence a first choice method for the identification and genus or species classification of prokaryotic systems (Kim & Chun, 2014).

Due to genetic homology between certain organisms belonging to different genera, the 16S rRNA approach is sometimes less valuable in discriminating closely related species such as the *L. plantarum* and *L. casei* group. Therefore, a further distinction of bacteria genera using species-specific PCRs may be useful identifying closely related species in future studies (Lv *et al.*, 2016) Wang, Jordan and Mayer (2015) suggested distinguishing strains showing more than 99% similarity in 16S rRNA gene sequences by DNA-DNA hybridization, for example, *B. globisporus* and *B. psychrophilus* strains that showed 99.5% sequence similarity in 16S rRNA genes, but only 23 – 50% gene similarity by DNA-DNA hybridization (Wisotzkey, Jurtshuk JR, Fox, Deinhard & Poralla, 1992). This approach is, however, labour-intensive and prone to error (Gevers, Cohan, Lawrence, Spratt, Coenye, Feil, Stackebrandt, Van de Peer, Vandamme & Thompson, 2005).

4.1.4 Estimation of bioaccessible minerals by *in vitro* dialysability assay

The *in vitro* dialysability assay was used to determine the amount of dialysable minerals from the fermented beverage samples. The technique gives an estimate of the amount of minerals released from food and are available for absorption with a physiological endpoint (Etcheverry, Grusak & Fleige, 2012). This is achieved through a simulated two-step human digestion process (gastric and intestinal stage) and using the dialysis membrane to mimic the interaction between the intestinal membrane with minerals (Sandberg, 2005; Fioravanti, Milani, de Paiva & Morgano, 2020).

Compared to *in vivo* models which are expensive and with complex ethical questions, *in vitro* methods have the advantage of being fast and less expensive as well as the absence of ethical restrictions (Minekus, Alminger, Alvito, Ballance, Bohn, Bourlieu, Carriere, Boutrou, Corredig & Dupont, 2014; Brodkorb, Egger, Alminger, Alvito, Assunção, Ballance, Bohn, Bourlieu-Lacanal, Boutrou & Carrière, 2019). Experimental variables are also easily controlled with *in vitro* models as opposed to human subjects (Sandberg, 2005). However, the method can be limited as it cannot ascertain transport and absorption kinetics in the human host (Fioravanti *et al.*, 2020). Caco-2 cells may be employed for uptake studies to predict mineral bioavailability (Etcheverry *et al.*, 2012; Minekus *et al.*, 2014) depending on the study hypothesis. An extension to the *in vitro* dialysability assay has been proposed, which includes a “continuous-flow dialysis system” using a “hollow-fibre system”. This modified system is thought to lead to better estimation of *in vivo* bioavailability (Wolters, Schreuder, Van Den Heuvel, Van Lonkhuijsen, Hermus & Voragen, 1993; Etcheverry *et al.*, 2012).

Another drawback that is known for the *in vitro* dialysability assay is the variation in the digestion parameters in the different models used in different studies reported in the literature. This makes it difficult to deduce general findings by comparing results across several (different) studies. Also, the sensitivity of enzyme activity, which is easily altered by pH and concentration of salts, such as calcium, has an impact on digestion and release of minerals from the food matrix. This makes comparison of results from different studies even more difficult especially if the models of the different studies apply different gastric pH (Minekus *et al.*, 2014). Dynamic digestion methods that mimic physiological conditions *in vivo***,** including the oral, gastric, intestinal phase, and sometimes intestinal fermentation have been developed. The methods consider amongst other factors changes in the concentration of digestive enzymes, digestion time, pH, and salt concentration. Other sophisticated models such as the Dutch TNO

gastrointestinal tract model can further simulate complex processes, such as transport of the digest, variable enzyme concentration, and changes in pH over time (Minekus, 1998; Minekus *et al.*, 2014). Although these models give a holistic view of the digestion process, they are expensive hence not always practically feasible where resources are constrained.

4.1.5 Determination of total phenolics by the Folin-Ciocalteu method

The Folin-Ciocalteu (F-C) method was used to quantity phenolic compounds. The method relies on a redox type of chemical reaction in which electrons are transferred from phenolic compounds to phosphotungstic/ phosphomolybdic acid complexes at alkaline pH. The reduction of the F-C reagent facilitates a colour change which can then be measured at 760 nm (Singleton, Orthofer & Lamuela-Raventós, 1999; Ainsworth & Gillespie, 2007). Although the method is simple, highly reproducible, and routinely used for the analysis of plant extracts, the sensitivity of F-C reagent to phenols precisely is questionable (Dai & Mumper, 2010). Everette, Bryant, Green, Abbey, Wangila and Walker (2010) did a thorough study on the reactivity of F-C reagent towards over 80 non-phenolic compounds. They found out that thiols, vitamins, amino acids, unsaturated fatty acids, organic acids, and some inorganic ions, amongst others, were reactive to F-C reagent which may skew results for TPC determinations. Unless calculations take into account interferences from non-phenolic reducing substrates, the reducing power of plant extracts cannot always be attributed to phenolic compounds but also other constituents of the food matrix that are capable of reducing the F-C reagent while being oxidised (Ford, Theodoridou, Sheldrake & Walsh, 2019). The reducing power of Maillard reaction products (MRPs) in processed foods has also been reported (Nooshkam, Varidi & Bashash, 2019). Possibly, MRPs could have been formed during heat treatment of slurries which may mean that values obtained in this study are not absolute rather an approximation of the phenolic content of samples. Combining the F-C assay with more specific methods such as liquid chromatography-mass spectrometry (LCMS) or high-performance liquid chromatography (HPLC) gives a better and more accurate profile of phenolic compounds (Van der Merwe, 2017).

4.1.6 The ABTS method to determine antioxidant activity

The ABTS (2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radical cation decolorization assay was used to determine the free radical scavenging capacity of antioxidants in finger millet-based beverages. When present, antioxidant molecules reduce $ABTS^{\bullet+}$ which diminishes the colour of the radical in solution from blueish-green to colourless. The extent of

decolourization relative to the Trolox standard is measured using a spectrophotometer and the Trolox equivalent antioxidant capacity (TEAC) values of extracts are calculated (Miller, Rice-Evans, Davies, Gopinathan & Milner, 1993). Due to the simplicity of this method and solubility of the radical in media with both organic and inorganic solvents, it is a widely used technique for determining the antioxidant capacity of both hydrophilic and lipophilic extracts (Prior, Wu & Schaich, 2005). The method is also environmentally friendly and economic as it can be adapted to 96-well microplates thus using a small volume of chemical reagents. However, the radical does not occur naturally in living systems which means results may not be representative of the antioxidant activities in biological systems, including humans (San Miguel-Chávez, 2017). In future studies of antioxidant properties, it would be useful to combine the ABTS radical scavenging assay with other antioxidant assays that proceed by different mechanisms such as the Oxygen Radical Absorption Capacity (ORAC) assay, Ferric Reducing Antioxidant Power (FRAP) assay, and others which are more simulative of living biological systems.

4.2 Key research findings

The results of this study showed that the fermentation process was mediated by a consortium of LAB species belonging to the genera *Enterococcus*, *Lactobacillus*, *Lactococcus*, and *Weissella*. Based on the initial identification of isolates, over 50% corresponded with *Weissella confusa* which were predominant mostly at the end of fermentation followed by E. *casseliflavus*, *E. faecium*, *L. plantarum*, *L. amylophilus*, *L. paracasei* ssp. *paracasei* and other LAB species which occurred at less than 2% of the total LAB abundance especially at the onset of fermentation. These include *L. salivarius*, *L. reuteri*, *L. mali*, *L. delbrueckii* ssp. *bulgaricus*, *L. nagelii*, *L. sakei*, *L. coryniformis* ssp. *coryniformis*, *L. malefermentans*, and others (Figure 3.7). The low abundance of *Lactobacilli* in cereal-based beverages prepared on a laboratory scale compared to products prepared under artisanal conditions has been reported (Diaz *et al.*, 2019). Although the raw materials used seemed to influence the bacterial diversity and succession, *E. casseliflavus* was isolated both at the onset and end of fermentation in all the formulations while *W. confusa* dominated only at the end especially in the finger millet and rice formulation where it corresponded with 71% of the isolates.

Genus level identification based on 16S rRNA amplicon sequencing revealed the dominance of *Weissella* in concentrations between 56.1 – 64.31% after 14 h of fermentation. After *Weissella*, the genera *Enterococcus* was also positively identified and increased from 5.25 –

22.28% throughout fermentation. Interestingly with this data, *Lactococcus* activity was detected after 14 h of fermentation only in the formulation with added maize starch slurry (Figure 3.9). In this study, species-level identification with 16S rRNA gene sequencing was less definitive which has been reported in other studies especially where closely related species of *Lactobacillus* are present hence the advantage of applying both culture-dependent and independent techniques where detailed insights into bacteria diversity and succession during fermentation is sought (Madoroba, 2011; Milanović, Osimani, Garofalo, Belleggia, Maoloni, Cardinali, Mozzon, Foligni, Aquilanti & Clementi, 2020). Similarly, Madoroba (2011) observed that use of culture-dependent techniques allowed for the identification of more bacteria compared to culture-independent techniques. This can happen when the bacteria occur in numbers below the detection limit of molecular techniques. From these results, *Weissella* ssp. appear to be well adapted to the food matrix and playing a significant role in the spontaneous fermentation of finger millet-based slurries which raises a hypothesis for its potential use in the preparation of starter cultures for controlled and standardised cereal-based fermentations.

Table 4.1. Summary of LAB isolates from spontaneously fermented finger millet-based slurries with or without added starch

Genera	**Respective species**	
Enterococcus	*E. casseliflavus*	*E. hermanniensis*
	E. faecium	*E. mundtii*
Lactobacillus	*L. acidipiscis*	*L. paracasei* ssp. *paracasei*
	L. amylophilus	*L. reuteri*
	L. coryniformis ssp.	*L. sakei*
	coryniformis	*L. salivarius*
	L. delbrueckii ssp. *bulgaricus*	*L. kimchi*
	L. delbrueckii ssp. *lastis*	*L. malefermentans*
	L. fructivorans	*L. mali*
	L. graminis	*L. nagelii*
	L. plantarum	
Lactococcus	*L. lactis*	*L. lastis* ssp. *tructae*
Weissella	*W. confusa*	

LAB have been implicated in the fermentation of several African cereal-based products (Madoroba, 2011). The overall quality of fermented cereal-based products is attributed to the transformation of the food matrix caused by LAB metabolic activities and sometimes yeasts, which in turn leads to microbial succession during fermentation as only organisms well adapted to low pH can survive (Houngbédji *et al.*, 2018). In a study by Olaniran, Abiose, Adeniran, Gbadamosi and Iranloye (2020) on the effect of co-fermentation of a cereal-based product (*Ogi*) on microbiome, mostly *Lactobacilli* were prominent during fermentation. Other LAB species namely, *L. coryniformis*, *L. fermentum*, *L. brevis*, *L. paracasei*, *L. paralimentarius W. confusa*, and *W. cibaria*, have also been isolated in *boza*, *ting*, and other wheat-based soured products (Madoroba, 2011; Milanović *et al.*, 2020). The antimicrobial role of LAB against pathogenic species or other plant-specific microbes during fermentation has been reported (Fessard & Remize, 2017). As observed in this study, 16S rRNA gene sequencing data revealed a decline in the population of bacteria belonging to the phyla Proteobacteria such as the genera *Sphingomonas*, *Rhizobium*, and *Enterobacter* from roughly 60.67% to less than 12%, with the dominance of LAB (3.19 Log cfu/ml to 9.42 Log_{10} cfu/ml) as shown in Figure 3.5. This can be linked to the acidification of slurries due to carbohydrate fermentation by LAB, which has an inhibitory effect on microbes not involved in the fermentation process including pathogenic species (Nout & Motarjemi, 1997; Madoroba, 2011).

Overall, the addition of exogenous maize or rice starch during fermentation did not affect the microbiological profile and diversity of finger millet-based slurries. The study, however, provided information about the microbial diversity (Table 4.1) and succession dynamics of fermented finger millet slurries with or without starch. This information can be effectively used in the design of suitable starter cultures for controlled fermentation of cereal-based products with enhanced nutritional and functional properties.

In this research, the addition of an exogenous starch source during fermentation did not affect protein quality, that is, *in vitro* protein digestibility (IVPD) and reactive (available) lysine. However, fermentation as a processing step, improved IVPD of finger millet-based beverages especially cooked samples that had higher IVPD than cooked, but unfermented counterparts (Table 3.10).It was noteworthy to observe that fermentation helped alleviate the negative effects of cooking on IVPD of slurries even though this was not clearly visible with reactive lysine content. The positive effects of fermentation on IVPD of cooked beverages observed in this study can be ascribed to the pre-digestion of finger millet storage proteins (prolamines) by

microbial and endogenous proteases into peptides and free amino acids. These are more soluble and accessible to proteolytic enzymes (Chavan *et al.*, 1988; Day & Morawicki, 2018). Also, the reduction in antinutritional factors during fermentation, precisely phytate in this research, due to activation of endogenous phytases at low pH may have improved protein digestibility (Nout & Motarjemi, 1997; Nkhata *et al.*, 2018). Phytate is well known to form insoluble complexes with protein hindering its susceptibility to pepsin attack (Kumar *et al.*, 2010). The phytate phosphoric groups interact with cationic groups of amino acids via electrostatic linkages (binary binding) or indirectly via multivalent bridges (tertiary binding) involving cations such as calcium (Ravindran *et al.*, 1999; Selle *et al.*, 2000; Kumar *et al.*, 2010; Morales *et al.*, 2016). Hydrolysis of such complexes improves the bioavailability of plant proteins for digestion into soluble forms as illustrated in Figure 4.1.

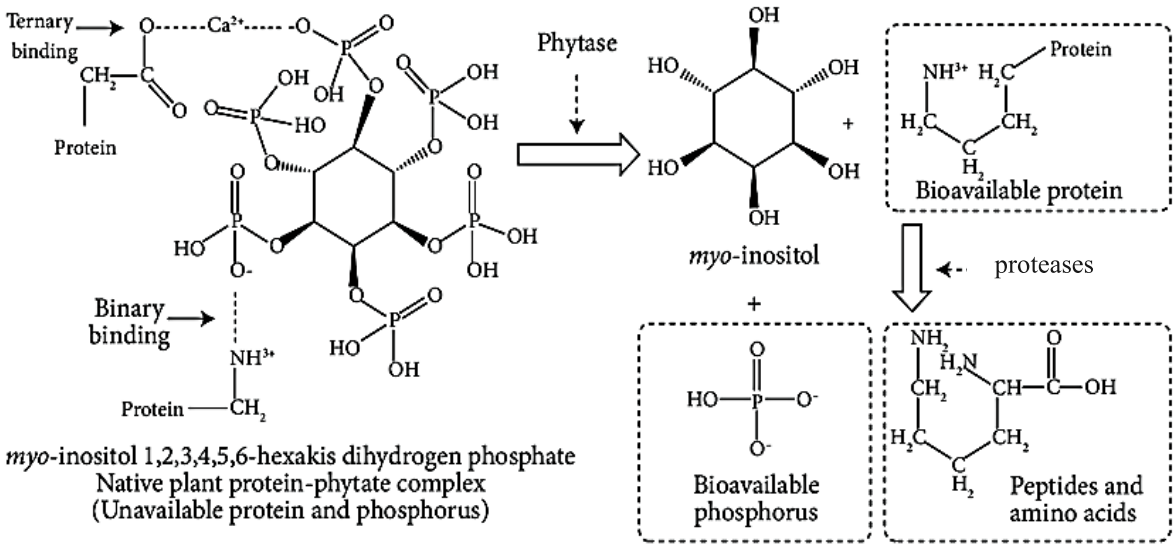


Figure 4.1. Proposed model to illustrate direct and indirect binding of proteins to phytate phosphoric groups and subsequent disruption of linkages in the presence of phytase. Adapted from Morales, Marquez, Hernández and Moyano (2016)

Concerning mineral bioaccessibility, beverages with added starch slurry had higher overall bioaccessible iron and zinc as well as a higher overall percentage bioaccessible iron and zinc compared to finger millet-based beverages without added starch. Presumably, a complex may have been formed between previously bound mineral ions and hydroxyl groups of starch whose release during digestion improved the overall bioaccessibility of cations *in vitro* as illustrated in Figure 4.1. According to Łabanowska *et al.* (2013), hydroxyl groups of starch, in its granular,

native, or pregelatinised form, can act as coordination sites for mineral ions such as Fe and Zn. Upon digestion, the starch: cation complexes can be released, which may result (Sigdel, 2019) in improved mineral bioaccessibility (Figure 4.2).

Interestingly, the phytate content of soured beverages with added starch slurry was relatively lower than that of finger millet-only samples. Since there is a proven link between starch concentration and microbial activity (Harlow *et al.*, 2015), it is possible that the addition of extra starch enhanced microbial activity during fermentation subsequently better microbial enzyme (phytase) activity and effective hydrolysis of phytate (Gibson *et al.*, 2010). While the decrease in phytate content of soured beverages with added starch was sufficient to improve zinc bioaccessibility, soured beverages with added starch had lower bioaccessible iron than unfermented counterparts, though higher than that of finger millet beverages (fermented and unfermented) without added starch. This suggests the chelation of iron by other non-phytate compounds in the fermentation medium, such as phenolic compounds, which were higher in fermented beverages with added starch. Phenolic compounds can chelate iron through hydroxyl groups reducing its overall bioaccessibility. Although the reason for the different effects of phenolic compounds on iron and zinc is unclear, it can be speculated that the phenolic compounds present had a higher iron-chelating affinity than zinc. (Afsana, Shiga, Ishizuka & Hara, 2004). Also, since iron concentration was approximately 8- 10 times higher than zinc in the study (Table 3.8), it can be speculated that there was more iron available to react with phenolic compounds compared to zinc (concentration effect), hence lower bioaccessible iron.

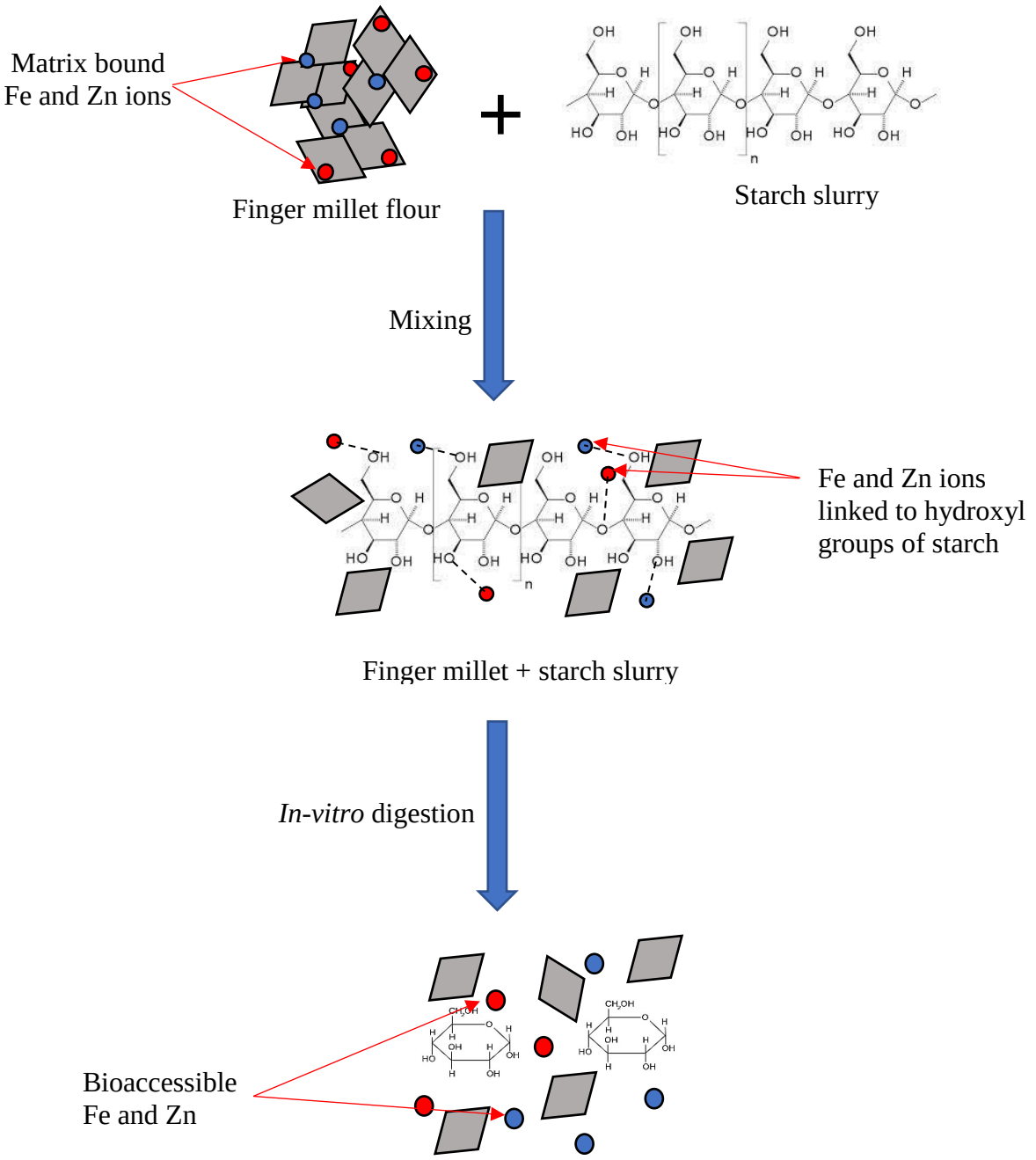


Figure 4.2. A proposed diagrammatic model of starch: mineral ion complexation in finger millet-based beverages

Another interesting observation from this study was the increase in total phenolic content and free radical scavenging activity of soured finger millet-based beverages with added starch, whereas finger millet only samples showed a decrease in both total phenolics and radical scavenging activity as shown in Tables 3.11 and 3.12. As mentioned earlier, the addition of extra starch may have improved fermentation efficiency and the mobilisation of hydrolytic enzymes, such as cell wall degrading esterases. These can enhance the release of phenolic

compounds which are cross-linked to cell wall matrices by cleaving ester bonds making phenolic compounds more soluble and extractable in solution (Duodu, 2014; Adebo & Gabriela Medina-Meza, 2020). Also important in that regard, are proteases, amylases, and xylanases which are activated endogenously at low pH or secreted by fermentation microflora. These enzymes contribute to the modification of cell wall matrixes leading to the liberation of bound phenolics into more assayable free forms hence an increase in total phenolics and antioxidant capacity as observed in this work (Adebo & Gabriela Medina-Meza, 2020). The increase antioxidant capacity would be attributed to the fact that free forms of the phenolic compounds have higher antixodant activity than the bound forms of the phenolic compounds.

Apart from their beneficial role in health as antioxidants, phenolic compounds are also important antinutritional factors and can negatively affect mineral bioaccessibility in food. In this study, the increase in the total phenolic content of soured finger millet-based beverages with added starch was accompanied by a decrease in percentage and amount of bioaccessible iron, whilst zinc bioaccessibility was not negatively affected. More specifically, soured beverages with added exogenous starch had relatively higher percentage bioaccessible zinc than unfermented counterparts. The high content of phenolic compounds in soured beverages with added starch may have contributed to the decrease in iron bioaccessibility. This is because phenolic compounds can form insoluble complexes with iron via catechol or gallolyl groups rendering the mineral unavailable for absorption (Brune, Hallberg & Skanberg, 1991; Van der Merwe, 2017). The cation-binding capacity of phenolic compounds differs based on phenolic groups. For example, catechol groups, which are common in cereal grains and gallolyl groups, have a high affinity for iron (Towo *et al.*, 2006). This motivates the proposed hypothesis in this research that the phenolic compounds present may have had a stronger chelating affinity for iron than for zinc.

Thermal processing of finger millet-based slurries did not have any observable effect on total phenolic content, however, caused a decrease in antioxidant capacity of samples. During processing, phenolic compounds can interact with other non-phenolic compounds in the food system, mainly macromolecules such as proteins (Figure 4.3), in such a manner that they are protected from degradation. In such cases, no observable changes in the phenolic content of a food system may be detected after processing (Duodu, 2014). The decrease in antioxidant activity (AA) after cooking can be due to the existence of other heat unstable bioactive

constituents, such as bioactive peptides, that contribute to the AA of finger millet-based slurries (Martinez-Villaluenga *et al.*, 2017; Adebo & Gabriela Medina-Meza, 2020). Their degradation during thermal processing can contribute to the decrease in AA observed.

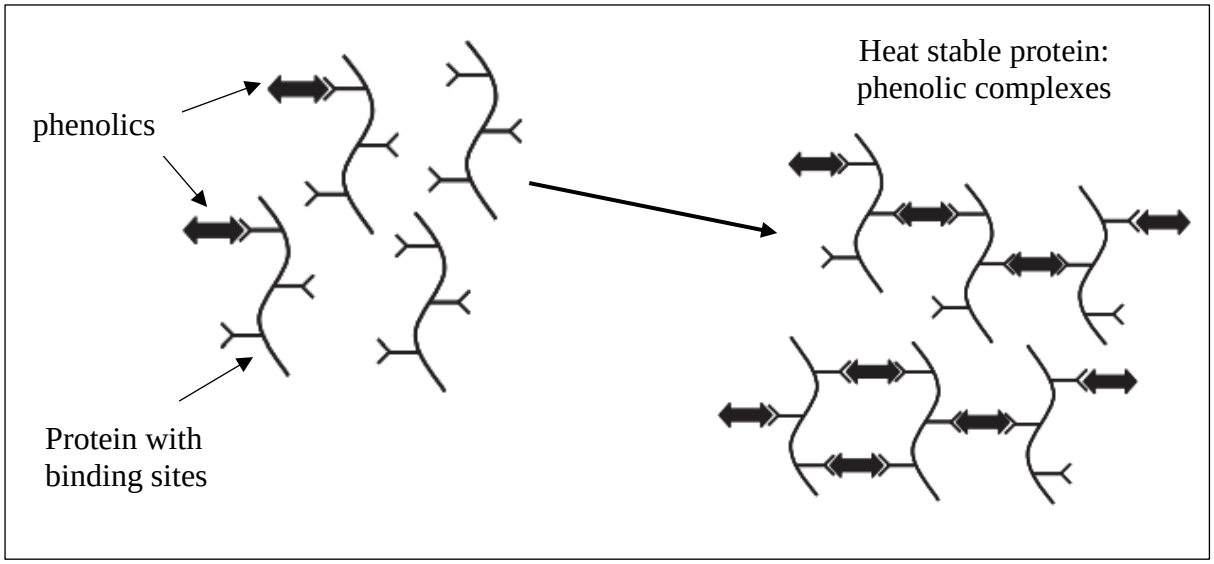


Figure 4.3. Illustration of protein-phenolic interactions during thermal processing. Modified from Le Bourvellec & Renard (2012)

Overall, the addition of exogenous starch during spontaneous fermentation of finger millet-based beverages is a practical, less complex, and cost-effective approach that can be used by low-income communities especially in Africa, where diets are largely cereal-based, to enhance phenolic content and antioxidant properties of whole-grain products. Consumption of these products, in addition to others, can help minimize the occurrence of non-communicable diseases related to free radical cellular damage.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

Finger millet fermentations, with or without added exogenous starch are dominated by lactic acid bacteria (LAB) specifically organisms belonging to the genera *Enterococcus*, *Lactobacillus*, *Lactococcus*, and *Weissella*. The species *Weissella confusa* is predominant at the end of fermentation followed by *Enterococcus casseliflavus*, *Enterococcus hermanniensis*, *Lactobacillus acidipiscis*, *Lactobacillus salivarius*, *Lactococcus lactis*, *Lactobacillus fructivorans*, and *Lactobacillus lactis* ssp. *tructae*. Although some plant-specific and potentially pathogenic organisms belonging to the genera *Sphingomonas*, *Rhizobium*, and *Enterobacter* are active at the onset of fermentation, their abundance declines with some genera not detected after 14 h of fermentation. The reduction of these bacterial populations is a result of the antimicrobial role of LAB as they create an acidic environment during fermentation by converting carbohydrates to organic acids. The low pH environment is selective for acid-tolerant bacteria. The predominance of *W. confusa* supports its use as a starter culture to control the nutritional and health-promoting properties of fermented cereal-based products as well as ensure their safety for large-scale production.

The addition of exogenous starch to finger millet-based beverages does not affect protein quality (IVPD and reactive lysine). Although cooking reduces the IVPD and reactive lysine content of beverages, souring by spontaneous fermentation is effective in alleviating the negative effects of cooking as fermented and cooked beverages have greater IVPD compared to unfermented and cooked beverages. The pre-digestion of storage proteins and hydrolysis of bound proteins by microbial and cereal endogenous enzymes activated at low pH during fermentation improves the solubility and accessibility of proteins to proteolytic enzymes. This improves the overall digestibility of proteins hence the higher IVPD of fermented beverages.

Finger millet fermentation with added exogenous starch (maize) significantly improves iron and zinc bioaccessibilities. This is probably due to the complexation of starch hydroxyl groups (OH) with iron and zinc forming starch: cation complexes which lend themselves to being easily broken down during *in vitro* digestion leading to improved mineral bioaccessibility. Furthermore, the phytate content of fermented samples with added starch slurry is much lower than that of finger millet samples without added starch. The addition of extra starch enhances the activity of fermentation microflora which is followed by better mobilisation of microbial

enzymes such as phytases which hydrolyse phytate to lower inositol phosphates hence the reduced phytate content.

As this research demonstrates, fermented and cooked finger millet-based beverages are important sources of digestible proteins. Also, the addition of starch during fermentation of finger millet-based beverages enhances the amount of bioaccessible iron and zinc. Therefore, fermented cereal-based foods can potentially be used to enhance the nutritional quality of Sub-Saharan African (SSA) diets towards combating protein malnutrition and deficiencies of minerals.

Lactic acid fermentation of finger millet-based beverages with added exogenous starch improves phenolic content and antioxidant properties. The addition of extra starch could enhance the activity of fermenting microflora resulting in better activation and mobilisation of microbial hydrolytic enzymes such as esterases which break down the cell wall matrix leading to the release of bound phenolics. This increases the phenolic content and antioxidant properties of beverages. Cooking does not affect total phenolic content, however, significantly reduces the antioxidant properties of beverages. This is mainly due to the presence of other heat unstable non-phenolic compounds which contribute to the antioxidant properties of fermented extracts such as bioactive peptides which are released from parent proteins during fermentation through microbial and/or endogenous enzyme activity. The high phenolic content and substantial radical scavenging capacity of fermented finger millet-based beverages with added starch substantiate them as important functional foods that can practically contribute to the alleviation of chronic diseases in which reactive oxygen species are implicated like cardiovascular diseases and some types of cancer.

Future research into microbial properties of finger millet-based beverages using 16S rRNA gene studies should consider functional annotation of microbial genes using gene reconstruction methods such as Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) to predict taxonomy-function interactions. This will provide a more impactful understanding of the taxonomic profile of the metagenome of fermented finger millet slurries and the symbiotic biochemical interactions that contribute to the nutritional and functional properties of the final product. Further, profiling and quantification of individual phenolic compounds in the finger millet-based beverages using analytical techniques such as Liquid Chromatography- mass spectrometry (LC-MS) are recommended to

explain the antioxidant capacity of slurries. Also, the physiological relevance of the antioxidant potential results can be established by measuring the inhibitory potential of beverage extracts *in vitro* against radical-induced deoxyribonucleic acid [DNA] damage (inhibition of oxidative DNA damage mechanism). Finally, the Caco-2 absorption model can be carried out to assess bioaccessibility and absorption dynamics of iron and zinc by human intestinal cells.

CHAPTER 6: REFERENCES

Acosta-Estrada, B.A., Gutiérrez-Uribe, J.A. & Serna-Saldívar, S.O. 2014. Bound phenolics in foods, a review. *Food Chemistry*, 152:46-55.

Adebo, O.A. & Gabriela Medina-Meza, I. 2020. Impact of fermentation on the phenolic compounds and antioxidant activity of whole cereal grains: A mini review. *Molecules*, 25(4):927. http://dx.doi.org/10.3390/molecules25040927

Adebo, O.A., Njobeh, P.B. & Kayitesi, E. 2018. Fermentation by Lactobacillus fermentum strains (singly and in combination) enhances the properties of ting from two whole grain sorghum types. *Journal of Cereal Science*, 82:49-56.

Adelakun, O.E. & Oyelade, O.J. 2011. Potential use of okra seed (Abelmoschus esculentus moench) flour for food fortification and effects of processing. In: Preedy, V.R., Watson, R.R. & Patel V.B. (Eds.), *Flour and Breads and their Fortification in Health and Disease Prevention*. Academic Press, pp. 205-212. https://doi.org/10.1016/B978-0-12-380886-8.10019-4

Adeyanju, A.A., Kruger, J., Taylor, J.R.N. & Duodu, K.G. 2019. Effects of different souring methods on the protein quality and iron and zinc bioaccessibilities of non-alcoholic beverages from sorghum and amaranth. *International Journal of Food Science & Technology*, 54(3):798-809.

Adom, K.K. & Liu, R.H. 2002. Antioxidant activity of grains. *Journal of Agricultural & Food Chemistry*, 50(21):6182-6187.

Afsana, K., Shiga, K., Ishizuka, S. & Hara, H. 2004. Reducing effect of ingesting tannic acid on the absorption of iron, but not of zinc, copper and manganese by rats. *Bioscience, Biotechnology & Biochemistry*, 68(3):584-592.

Ainsworth, E.A. & Gillespie, K.M. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature Protocols*, 2(4):875-877.

Alavi, S., Mazumdar, S.D. & Taylor, J.R.N. 2019. In: Taylor, J.R.N & Duodu K.G. (Eds.), Modern convenient sorghum and millet food, beverage and animal feed products and their technologies. *Sorghum and Millets*: *Chemistry, Technology and Nutritional Attributes*.

Cambridge: AACC International Press and Elsevier. pp. 293-329. https://doi.org/10.1016/B978-0-12-811527-5.00010-1

Alberts, M., Urdal, P., Steyn, K., Stensvold, I., Tverdal, A., Nel, J.H. & Steyn, N.P. 2005. Prevalence of cardiovascular diseases and associated risk factors in a rural black population of South Africa. *European Journal of Cardiovascular Prevention & Rehabilitation*, 12(4):347-354.

Ali, A., Wani, T.A., Wani, I.A. & Masoodi, F.A. 2016. Comparative study of the physico-chemical properties of rice and corn starches grown in Indian temperate climate. *Journal of the Saudi Society of Agricultural Sciences*, 15(1):75-82.

Alka, S., Neelam, Y. & Shruti, S. 2012. Effect of fermentation on physicochemical properties and in vitro starch and protein digestibility of selected cereals. *International Journal of Agricultural & Food Science*, 2(3):66-70.

Amoa-Awua, W. & Jakobsen, M. 1995. The role of Bacillus species in the fermentation of cassava. *Journal of Applied Bacteriology*, 79(3):250-256.

Anderson, M.J. 2014. Permutational multivariate analysis of variance (PERMANOVA). *Wiley Statsref: Statistics Reference Online*, 1-15. https://doi.org/10.1002/9781118445112.stat07841

Annor, G.A., Tyl, C., Marcone, M., Ragaee, S. & Marti, A. 2017. Why do millets have slower starch and protein digestibility than other cereals? *Trends in Food Science & Technology*, 66:73-83.

Antony Ceasar, S., Maharajan, T., Ajeesh Krishna, T., Ramakrishnan, M., Victor Roch, G., Satish, L. & Ignacimuthu, S. 2018. Finger millet [Eleusine coracana (L.) Gaertn.] improvement: current status and future interventions of whole genome sequence. *Frontiers in Plant Science*, 9:1054. https://doi.org/10.3389/fpls.2018.01054

Antony, U. & Chandra, T. 1998. Antinutrient reduction and enhancement in protein, starch, and mineral availability in fermented flour of finger millet (Eleusine coracana). *Journal of Agricultural & Food Chemistry*, 46(7):2578-2582.

Antony, U. & Chandra, T. 1999. Enzymatic treatment and use of starters for the nutrient enhancement in fermented flour of red and white varieties of finger millet (Eleusine coracana). *Journal of Agricultural & Food Chemistry*, 47(5):2016-2019.

Antony, U. & Chandra, T. 1997. Microbial population and biochemical changes in fermenting finger millet (Eleusine coracana). *World Journal of Microbiology & Biotechnology*, 13(5):533-537.

Apea-Bah, F.B., Minnaar, A., Bester, M.J. & Duodu, K.G. 2014. Does a sorghum–cowpea composite porridge hold promise for contributing to alleviating oxidative stress? *Food Chemistry*, 157:157-166.

Assohoun, M.C., Djeni, T.N., Koussémon-Camara, M. & Brou, K. 2013. Effect of fermentation process on nutritional composition and aflatoxins concentration of doklu, a fermented maize based food. *Food & Nutrition Sciences*, 4(11):1120-1127. doi: 10.4236/fns.2013.411146.

AwadElkareem, A.M. & Taylor, J.R. 2011. Protein quality and physical characteristics of kisra (fermented sorghum pancake-like flatbread) made from tannin and non-tannin sorghum cultivars. *Cereal Chemistry*, 88(4):344-348.

Ayodeji, B.D., Piccirillo, C., Ferraro, V., Moreira, P.R., Obadina, A.O., Sanni, L.O. & Pintado, M.M. 2017. Screening and molecular identification of lactic acid bacteria from gari and fufu and gari effluents. *Annals of Microbiology*, 67(1):123-133.

Bamidele, O., Duodu, K. & Emmambux, M. 2017. Encapsulation and antioxidant activity of ascorbyl palmitate with maize starch during pasting. *Carbohydrate Polymers*, 166:202-208.

Bao, J. 2019. Rice Starch. *College of Agriculture and Biotechnology, Zhejiang University, Hanzhou, China*, 55-108. https://doi.org/10.1016/B978-0-12-811508-4.00003-4

Baye, K., Guyot, J.-P., Icard-Vernière, C., Rochette, I. & Mouquet-Rivier, C. 2015. Enzymatic degradation of phytate, polyphenols and dietary fibers in Ethiopian injera flours: Effect on iron bioaccessibility. *Food Chemistry*, 174:60-67.

Baye, K., Mouquet-Rivier, C., Icard-Vernière, C., Picq, C. & Guyot, J.P. 2014. Changes in mineral absorption inhibitors consequent to fermentation of Ethiopian injera: implications for predicted iron bioavailability and bioaccessibility. *International Journal of Food Science & Technology*, 49(1):174-180.

BeMiller, J.N. 2018. *Carbohydrate Chemistry for Food Scientists*. 3rd Edition. Woodhead Publishing, Cambridge, USA.

Bennett, J.E., Stevens, G.A., Mathers, C.D., Bonita, R., Rehm, J., Kruk, M.E., Riley, L.M., Dain, K., Kengne, A.P. & Chalkidou, K. 2018. NCD Countdown 2030: Worldwide trends in non-communicable disease mortality and progress towards Sustainable Development Goal target 3.4. *The Lancet*, 392(10152):1072-1088.

Blandino, A., Al-Aseeri, M., Pandiella, S., Cantero, D. & Webb, C. 2003. Cereal-based fermented foods and beverages. *Food Research International*, 36(6):527-543.

Böhme, K., Fernández-No, I.C., Pazos, M., Gallardo, J.M., Barros-Velázquez, J., Cañas, B. & Calo-Mata, P. 2013. Identification and classification of seafood-borne pathogenic and spoilage bacteria: 16 S r RNA sequencing versus MALDI-TOF MS fingerprinting. *Electrophoresis*, 34(6):877-887.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M. & Asnicar, F. 2019. QIIME 2: Reproducible, Interactive, Scalable, and Extensible Microbiome Data Science. *Nature Biotechnology*, 37(8):852-857.

Bora, P., Das, P., Mohan, P. & Barthakur, A. 2018. Evaluation of hypolipidemic property of proso millet (Panicum miliaceum L.) in high fat diet induced hyperlipidemia in rats. *Journal Entomology and Zoology Studies*, 6(3):691-695.

Bora, P., Ragaee, S. & Marcone, M. 2019. Effect of parboiling on decortication yield of millet grains and phenolic acids and in vitro digestibility of selected millet products. *Food Chemistry*, 274:718-725.

Bourne, L.T., Lambert, E.V. & Steyn, K. 2002. Where does the black population of South Africa stand on the nutrition transition? *Public Health Nutrition*, 5(1a):157-162.

Bowman, J. & Firkins, J. 1993. Effects of forage species and particle size on bacterial cellulolytic activity and colonization in situ. *Journal of Animal Science*, 71(6):1623-1633.

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., Bohn, T., Bourlieu-Lacanal, C., Boutrou, R. & Carrière, F. 2019. INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols*, 14(4):991-1014.

Brune, M., Hallberg, L. & Skanberg, A.-B. 1991. Determination of iron-binding phenolic groups in foods. *Journal of Food Science*, 56(1):128-131.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. & Holmes, S.P. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods,* 13(7):581-583. https://doi.org/10.1038/nmeth.3869

Campbell-Platt, G. 1994. Fermented foods—a world perspective. *Food Research International*, 27(3):253-257.

Caplice, E. & Fitzgerald, G.F. 1999. Food fermentations: role of microorganisms in food production and preservation. *International Journal of Food Microbiology,* 50(1-2):131-149.

Caporasoa, J.G., Lauberb, C.L., Waltersc, W.A., Berg-Lyonsb, D., Lozuponea, C.A., Turnbaughd, P.J., Fierer, N. & Knight, R. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences of the United States of America,* 108:4516-4522.

Castellano, P., Belfiore, C., Fadda, S. & Vignolo, G. 2008. A review of bacteriocinogenic lactic acid bacteria used as bioprotective cultures in fresh meat produced in Argentina. *Meat Science,* 79(3):483-499.

Chakravorty, S., Bhattacharya, S., Chatzinotas, A., Chakraborty, W., Bhattacharya, D. & Gachhui, R. 2016. Kombucha tea fermentation: Microbial and biochemical dynamics. *International Journal of Food Microbiology,* 220:63-72.

Chandra, D., Chandra, S. & Sharma, A. 2016. Review of Finger millet (Eleusine coracana (L.) Gaertn): a power house of health benefiting nutrients. *Food Science & Human Wellness,* 5(3):149-155.

Chandrasekara, A. & Shahidi, F. 2011a. Antiproliferative potential and DNA scission inhibitory activity of phenolics from whole millet grains. *Journal of Functional Foods,* 3(3):159-170.

Chandrasekara, A. & Shahidi, F. 2010. Content of insoluble bound phenolics in millets and their contribution to antioxidant capacity. *Journal of Agricultural & Food Chemistry,* 58(11):6706-6714.

Chandrasekara, A. & Shahidi, F. 2011b. Determination of antioxidant activity in free and hydrolyzed fractions of millet grains and characterization of their phenolic profiles by HPLC-DAD-ESI-MSn. *Journal of Functional Foods,* 3(3):144-158.

Chavan, U., Chavan, J. & Kadam, S. 1988. Effect of fermentation on soluble proteins and in vitro protein digestibility of sorghum, green gram and sorghum-green gram blends. *Journal of Food Science*, 53(5):1574-1575.

Cheng, Y.-Y. & Tsai, T.-H. 2016. Analysis of Sheng-Mai-San, a Ginseng-containing multiple components traditional Chinese herbal medicine using liquid chromatography tandem mass spectrometry and physical examination by electron and light microscopies. *Molecules*, 21(9):1159. https://doi.org/10.3390/molecules21091159

Chiang, A.N., Wu, H.L., Yeh, H.I., Chu, C.S., Lin, H.C. & Lee, W.C. 2006. Antioxidant effects of black rice extract through the induction of superoxide dismutase and catalase activities. *Lipids*, 41(8):797-803.

Coulin, P., Farah, Z., Assanvo, J., Spillmann, H. & Puhan, Z. 2006. Characterisation of the microflora of attiéké, a fermented cassava product, during traditional small-scale preparation. *International Journal of Food Microbiology*, 106(2):131-136.

Da Silva, L.S., Taylor, J. & Taylor, J.R.N. 2011. Transgenic sorghum with altered kafirin synthesis: kafirin solubility, polymerization, and protein digestion. *Journal of Agricultural & Food Chemistry*, 59(17):9265-9270.

Dai, J. & Mumper, R.J. 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10):7313-7352.

Day, C.N. & Morawicki, R.O. 2018. Effects of fermentation by yeast and amylolytic lactic acid bacteria on grain sorghum protein content and digestibility. *Journal of Food Quality*, 2018:1-8. https://doi.org/10.1155/2018/3964392

Dayakar Rao, B., Bhaskarachary, K., Arlene Christina, G., Sudha Devi, G., Vilas, A.T. & Tonapi, A. 2017. Nutritional and health benefits of millets. *ICAR Indian Institute of Millets Research (IIMR) Rajendranagar, Hyderabad*, [Online] Available from: https://www.researchgate.net/profile/Gd_Arlene_Christina/publication/318921493_Nutritional_and_Health_Benefits_of_Millets/links/5985725e0f7e9b6c85333b78/Nutritional-and-Health-Benefits-of-Millets.pdf [accessed 29 April 2020].

De Benoist, B., Cogswell, M., Egli, I. & McLean, E.e. 2008. Worldwide Prevalence of Anaemia 1993-2005; WHO Global Database of Anaemia. Geneva: WHO Press. [Online] Available from:

https://apps.who.int/iris/bitstream/handle/10665/43894/9789241596657eng.pdf [accessed 27 February 2020].

De Boland, A.R., Garner, G.B. & O'Dell, B.L. 1975. Identification and properties of phytate in cereal grains and oilseed products. *Journal of Agricultural & Food Chemistry,* 23(6):1186-1189.

De Man, J., Rogosa, d. & Sharpe, M.E. 1960. A medium for the cultivation of lactobacilli. *Journal of Applied Bacteriology,* 23(1):130-135.

De Oliveira, F.C., Coimbra, J.S.d.R., de Oliveira, E.B., Zuñiga, A.D.G. & Rojas, E.E.G. 2016. Food protein-polysaccharide conjugates obtained via the Maillard reaction: A review. *Critical Reviews in Food Science & Nutrition,* 56(7):1108-1125.

Department of Health, M.R.C., OrcMacro. 2007. *South Africa demographic and health survey 2003.* [Online] Available from: https://dhsprogram.com/pubs/pdf/FR206/FR206.pdf [accessed 27 February 2020].

Devi, P.B., Vijayabharathi, R., Sathyabama, S., Malleshi, N.G. & Priyadarisini, V.B. 2014. Health benefits of finger millet (Eleusine coracana L.) polyphenols and dietary fiber: a review. *Journal of Food Science & Technology,* 51(6):1021-1040.

Dhankher, N. & Chauhan, B. 1987. Effect of temperature and period of fermentation on protein and starch digestibility (in vitro) of rabadi—A pearl millet fermented food. *Journal of Food Science,* 52(2):489-490.

Diaz, M., Kellingray, L., Akinyemi, N., Adefiranye, O.O., Olaonipekun, A.B., Bayili, G.R., Ibezim, J., du Plessis, A.S., Houngbédji, M. & Kamya, D. 2019. Comparison of the microbial composition of African fermented foods using amplicon sequencing. *Scientific Reports,* 9(1):1-8.

Dlamini, N.R., Taylor, J.R.N. & Rooney, L.W. 2007. The effect of sorghum type and processing on the antioxidant properties of African sorghum-based foods. *Food Chemistry,* 105(4):1412-1419.

Doelle, H.W. 2014. *Bacterial Metabolism.* 2nd Edition. Academic Press, New York.

Duodu, K.G. 2014. Effects of processing on phenolic phytochemicals in cereals and legumes. *Cereal Foods World,* 59(2):64-70.

Duodu, K.G., Minnaar, A. & Taylor, J.R.N. 1999. Effect of cooking and irradiation on the labile vitamins and antinutrient content of a traditional African sorghum porridge and spinach relish. *Food Chemistry,* 66(1):21-27.

Duodu, K., Nunes, A., Delgadillo, I., Parker, M., Mills, E., Belton, P. & Taylor, J.R.N. 2002. Effect of grain structure and cooking on sorghum and maize in vitro protein digestibility. *Journal of Cereal Science,* 35(2):161-174.

Duodu, K.G., Tang, H., Grant, A., Wellner, N., Belton, P. & Taylor, J.R.N. 2001. FTIR and solid State ^{13}C NMR spectroscopy of proteins of wet cooked and popped sorghum and maize. *Journal of Cereal Science,* 33(3):261-269.

Duodu, K.G., Taylor, J.R.N., Belton, P. & Hamaker, B. 2003. Factors affecting sorghum protein digestibility. *Journal of Cereal Science,* 38(2):117-131.

Duodu, K.G. 2019. Assaying sorghum nutritional quality. In: Zhao ZY., Dahlberg J. (Eds.), Sorghum. Methods in Molecular Biology, vol 1931. Humana Press, New York, NY, pp. 87-108.

Dykes, L. & Rooney, L. 2007. Phenolic compounds in cereal grains and their health benefits. *Cereal Foods World,* 52(3):105-111.

Edmonds, D.E., Abreu, S.L., West, A., Caasi, D.R., Conley, T.O., Daft, M.C., Desta, B., England, B.B., Farris, C.D. & Nobles, T.J. 2009. Cereal nitrogen use efficiency in sub Saharan Africa. *Journal of Plant Nutrition,* 32(12):2107-2122.

Ekpa, O., Palacios-Rojas, N., Kruseman, G., Fogliano, V. & Linnemann, A.R. 2019. Sub-Saharan African maize-based foods - processing practices, challenges and opportunities. *Food Reviews International,* 35(7):609-639.

El Hag, M.E., El Tinay, A.H. & Yousif, N.E. 2002. Effect of fermentation and dehulling on starch, total polyphenols, phytic acid content and in vitro protein digestibility of pearl millet. *Food Chemistry,* 77(2):193-196.

Elizaquível, P., Pérez-Cataluña, A., Yépez, A., Aristimuño, C., Jiménez, E., Cocconcelli, P.S., Vignolo, G. & Aznar, R. 2015. Pyrosequencing vs. culture-dependent approaches to analyze lactic acid bacteria associated to chicha, a traditional maize-based fermented beverage from Northwestern Argentina. *International Journal of Food Microbiology,* 198:9-18.

Etcheverry, P., Grusak, M.A. & Fleige, L.E. 2012. Application of in vitro bioaccessibility and bioavailability methods for calcium, carotenoids, folate, iron, magnesium, polyphenols, zinc, and vitamins B6, B12, D, and E. *Frontiers in Physiology*, 3:317. https://doi.org/10.3389/fphys.2012.00317

Evans, D.E., Collins, H., Eglinton, J. & Wilhelmson, A. 2005. Assessing the impact of the level of diastatic power enzymes and their thermostability on the hydrolysis of starch during wort production to predict malt fermentability. *Journal of the American Society of Brewing Chemists*, 63(4):185-198.

Everette, J.D., Bryant, Q.M., Green, A.M., Abbey, Y.A., Wangila, G.W. & Walker, R.B. 2010. Thorough study of reactivity of various compound classes toward the Folin− Ciocalteu reagent. *Journal of Agricultural & Food Chemistry*, 58(14):8139-8144.

Ezekiel, C.N., Ayeni, K.I., Ezeokoli, O.T., Sulyok, M., van Wyk, D.A., Oyedele, O.A., Akinyemi, O.M., Chibuzor-Onyema, I.E., Adeleke, R.A. & Nwangburuka, C.C. 2019. High-throughput sequence analyses of bacterial communities and multi-mycotoxin profiling during processing of different formulations of Kunu, a traditional fermented beverage. *Frontiers in Microbiology*, 9:3282. https://doi.org/10.3389/fmicb.2018.03282

Ezzati, M., Pearson-Stuttard, J., Bennett, J.E. & Mathers, C.D. 2018. Acting on non-communicable diseases in low-and middle-income tropical countries. *Nature*, 559(7715):507-516.

Faith, D.P. 1992. Conservation evaluation and phylogenetic diversity. *Biological Conservation*, 61(1):1-10.

FAOSTAT 2016. Food balance sheets. *FAO Statistical Databases: Rome, Italy*. http://faostat.fao.org/site/345/default.aspx [accessed 29 February 2020].

Feil, B. 2001. Phytic acid. *Journal of New Seeds*, 3(3):1-35.

Fessard, A. & Remize, F. 2017. Why are Weissella spp. not used as commercial starter cultures for food fermentation? *Fermentation*, 3(3):38. https://doi.org/10.3390/fermentation3030038

Figuerola, F., Hurtado, M.a.L., Estévez, A.M.a., Chiffelle, I. & Asenjo, F. 2005. Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. *Food Chemistry*, 91(3):395-401.

Fioravanti, M.I.A., Milani, R.F., de Paiva, E.L. & Morgano, M.A. 2020. Simple and fast ultrasound-assisted method for mineral content and bioaccessibility study in infant formula by ICP OES. *Analytical Methods,* 12(25):3225-3234.

Ford, L., Theodoridou, K., Sheldrake, G.N. & Walsh, P.J. 2019. A critical review of analytical methods used for the chemical characterisation and quantification of phlorotannin compounds in brown seaweeds. *Phytochemical Analysis,* 30(6):587-599.

Fox, G. 2018. Starch in Brewing Applications. *Starch in Food*: Elsevier. http://dx.doi.org/10.1016/B978-0-08-100868-3.00016-0

Freire, A.L., Ramos, C.L., de Almeida, E.G., Duarte, W.F. & Schwan, R.F. 2014. Study of the physicochemical parameters and spontaneous fermentation during the traditional production of yakupa, an indigenous beverage produced by Brazilian Amerindians. *World Journal of Microbiology & Biotechnology,* 30(2):567-577.

Friman, L., Höglund, H., Högberg, H.-E. & Agnemo, R. 2004. Tannin-iron impregnated thermomechanical pulp. Part I: Effects of extractions and heat on brightness. *Nordic Pulp & Paper Research Journal,* 19(2):229-236.

Fruhbeck, G., Alonso, R., Marzo, F. & Santidrián, S. 1995. A modified method for the indirect quantitative analysis of phytate in foodstuffs. *Analytical Biochemistry,* 225(2):206-212.

Fuller, R. 1989. Probiotics in man and animals. *Journal of Applied Bacteriology,* 66(5):365-378.

Gabaza, M., Muchuweti, M., Vandamme, P. & Raes, K. 2017. Can fermentation be used as a sustainable strategy to reduce iron and zinc binders in traditional African fermented cereal porridges or gruels? *Food Reviews International,* 33(6):561-586.

Gabaza, M., Shumoy, H., Louwagie, L., Muchuweti, M., Vandamme, P., Du Laing, G. & Raes, K. 2018a. Traditional fermentation and cooking of finger millet: Implications on mineral binders and subsequent bioaccessibility. *Journal of Food Composition and Analysis,* 68:87-94.

Gabaza, M., Shumoy, H., Muchuweti, M., Vandamme, P. & Raes, K. 2016. Effect of fermentation and cooking on soluble and bound phenolic profiles of finger millet sour porridge. *Journal of Agricultural & Food Chemistry,* 64(40):7615-7621.

Gabaza, M., Shumoy, H., Muchuweti, M., Vandamme, P. & Raes, K. 2018b. Iron and zinc bioaccessibility of fermented maize, sorghum and millets from five locations in Zimbabwe. *Food Research International,* 103:361-370.

Gadaga, T., Mutukumira, A., Narvhus, J. & Feresu, S. 1999. A review of traditional fermented foods and beverages of Zimbabwe. *International Journal of Food Microbiology*, 53(1):1-11.

Gaffa, T. & Gaffa, A. 2004. Microbial Succession During "Kunun Zaki" Production with Sorghum (Sorghum bicolor) Grains. *World Journal of Microbiology & Biotechnology*, 20(5):449-453.

Gevers, D., Cohan, F.M., Lawrence, J.G., Spratt, B.G., Coenye, T., Feil, E.J., Stackebrandt, E., Van de Peer, Y., Vandamme, P. & Thompson, F.L. 2005. Re-evaluating prokaryotic species. *Nature Reviews Microbiology,* 3(9):733-739.

Gibson, R.S., Bailey, K.B., Gibbs, M. & Ferguson, E.L. 2010. A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. *Food & Nutrition Bulletin,* 31(suppl 2):S134-S146.

Gilani, G.S., Xiao, C.W. & Cockell, K.A. 2012. Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. *British Journal of Nutrition,* 108(S2):S315-S332.

Greffeuille, V., Kayodé, A.P., Icard-Vernière, C., Gnimadi, M., Rochette, I. & Mouquet-Rivier, C. 2011. Changes in iron, zinc and chelating agents during traditional African processing of maize: effect of iron contamination on bioaccessibility. *Food Chemistry,* 126(4):1800-1807.

Greiner, R. & Konietzny, U. 2006. Phytase for food application. *Food Technology & Biotechnology,* 44(2):125-140

Greppi, A., Rantisou, K., Padonou, W., Hounhouigan, J., Jespersen, L., Jakobsen, M. & Cocolin, L. 2013. Yeast dynamics during spontaneous fermentation of mawè and tchoukoutou, two traditional products from Benin. *International Fournal of Food Microbiology,* 165(2):200-207.

Greyling, J.C. & Pardey, P.G. 2019. Measuring Maize in South Africa: the shifting structure of production during the twentieth century, 1904–2015. *Agrekon*, 58(1):21-41.

Guessas, B. & Kihal, M. 2004. Characterization of lactic acid bacteria isolated from Algerian arid zone raw goats\'milk. *African Journal of Biotechnology,* 3(6):339-342.

Gulati, P., Li, A., Holding, D., Santra, D., Zhang, Y. & Rose, D.J. 2017. Heating reduces proso millet protein digestibility via formation of hydrophobic aggregates. *Journal of Agricultural & Food Chemistry,* 65(9):1952-1959.

Gull, A., Jan, R., Nayik, G.A., Prasad, K. & Kumar, P. 2014. Significance of finger millet in nutrition, health and value added products: a review. *Journal of Environmental Science, Computer Science and Engineering & Technology),* 3(3):1601-1608.

Gupta, S., Cox, S. & Abu-Ghannam, N. 2010. Process optimization for the development of a functional beverage based on lactic acid fermentation of oats. *Biochemical Engineering Journal,* 52(2-3):199-204.

Hagerman, A. 2002. Condensed tannin structural chemistry. *Tannin Chemistry*, Miami University, USA.

Hagmeyer, S., Haderspeck, J.C. & Grabrucker, A.M. 2015. Behavioral impairments in animal models for zinc deficiency. *Frontiers in Behavioral Neuroscience,* 8:443. https://doi.org/10.3389/fnbeh.2014.00443

Harlow, B., Donley, T., Lawrence, L. & Flythe, M. 2015. Effect of starch source (corn, oats or wheat) and concentration on fermentation by equine faecal microbiota in vitro. *Journal of Applied Microbiology*, 119(5):1234-1244.

Hegde, P.S., Rajasekaran, N.S. & Chandra, T. 2005. Effects of the antioxidant properties of millet species on oxidative stress and glycemic status in alloxan-induced rats. *Nutrition Research,* 25(12):1109-1120.

Hofvendahl, K. & Hahn–Hägerdal, B. 2000. Factors affecting the fermentative lactic acid production from renewable resources1. *Enzyme & Microbial Technology,* 26(2-4):87-107.

Holzapfel, W. 2002. Appropriate starter culture technologies for small-scale fermentation in developing countries. *International Journal of Food Microbiology,* 75(3):197-212.

Holzapfel, W. 1997. Use of starter cultures in fermentation on a household scale. *Food Control,* 8(5-6):241-258.

Hoseney, R.C. 1994. Principles of Cereal Science and Technology. American Association of Cereal Chemists. *Inc., St. Paul*, MN.

Houngbédji, M., Johansen, P., Padonou, S.W., Akissoé, N., Arneborg, N., Nielsen, D.S., Hounhouigan, D.J. & Jespersen, L. 2018. Occurrence of lactic acid bacteria and yeasts at species and strain level during spontaneous fermentation of mawè, a cereal dough produced in West Africa. *Food Microbiology*, 76:267-278.

Hu, P., Zhao, H., Duan, Z., Linlin, Z. & Wu, D. 2004. Starch digestibility and the estimated glycemic score of different types of rice differing in amylose contents. *Journal of Cereal Science*, 40(3):231-237.

Humer, E. & Schedle, K. 2016. Fermentation of food and feed: A technology for efficient utilization of macro and trace elements in monogastrics. *Journal of Trace Elements in Medicine and Biology*, 37:69-77.

Hurrell 2004. Phytic acid degradation as a means of improving iron absorption. *International Journal for Vitamin & Nutrition Research*, 74(6):445-452.

Hurrell, R. & Egli, I. 2010. Iron bioavailability and dietary reference values. *The American Journal of Clinical Nutrition*, 91(5):1461S-1467S.

Issa, A.Y., Volate, S.R. & Wargovich, M.J. 2006. The role of phytochemicals in inhibition of cancer and inflammation: new directions and perspectives. *Journal of Food Composition & Analysis*, 19(5):405-419.

Jang, K.-S. & Kim, Y.H. 2018. Rapid and robust MALDI-TOF MS techniques for microbial identification: a brief overview of their diverse applications. *Journal of Microbiology*, 56(4):209-216.

Joy, E.J., Ander, E.L., Young, S.D., Black, C.R., Watts, M.J., Chilimba, A.D., Chilima, B., Siyame, E.W., Kalimbira, A.A. & Hurst, R. 2014. Dietary mineral supplies in Africa. *Physiologia Plantarum*, 151(3):208-229.

Joye, I. 2019. Protein digestibility of cereal products. *Foods*, 8(6):199. http://dx.doi.org/10.3390/foods8060199

Karovičová, Z.K.-J. & Kohajdova, J. 2007. Fermentation of cereals for specific purpose. *Journal of Food & Nutrition Research*, 46(2):51-57.

Kawashima, S., Hayashi, M., Takii, T., Kimura, H., Zhang, H.L., Nagatsu, A., Sakakibara, J., Murata, K., Oomoto, Y. & Onozaki, K. 1998. Serotonin derivative, N-(p-coumaroyl) serotonin, inhibits the production of TNF-α, IL-1α, IL-1β, and IL-6 by endotoxin-stimulated human blood monocytes. *Journal of Interferon & Cytokine Research,* 18(6):423-428.

Kayodé, A.P., Linnemann, A.R., Nout, M.J. & Van Boekel, M.A. 2007. Impact of sorghum processing on phytate, phenolic compounds and in vitro solubility of iron and zinc in thick porridges. *Journal of the Science of Food & Agriculture,* 87(5):832-838.

Khetarpaul, N. & Chauhan, B. 1990. Effect of germination and fermentation on in vitro starch and protein digestibility of pearl millet. *Journal of Food Science,* 55(3):883-884.

Kim, H., Pham, H. & Ziboh, V. 2001. Flavonoids differentially inhibit guinea pig epidermal cytosolic phospholipase A2. *Prostaglandins, Leukotrienes & Essential Fatty Acids (PLEFA),* 65(5-6):281-286.

Kim, J.-S., Kim, K.-J., Ma, W.C.J. & Chung, H.Y. 2007. Development of a Method to Quantify Lysine in Small Amount of Rice Grain. *Korean Journal of Sanitation,* 22(2):75-84.

Kim, M. & Chun, J. 2014. 16S rRNA gene-based identification of bacteria and archaea using the EzTaxon server. *Methods in Microbiology*, 41:61-74

Klein, G., Pack, A., Bonaparte, C. & Reuter, G. 1998. Taxonomy and physiology of probiotic lactic acid bacteria. *International Journal of Food Microbiology,* 41(2):103-125.

Krishnamoorthy, S., Coetzee, V., Kruger, J., Potgieter, H. & Buys, E.M. 2020. Dysbiosis signatures of fecal microbiota in South African infants with respiratory, gastrointestinal, and other diseases. *The Journal of Pediatrics,* 218:106-113.

Kumar, A., Metwal, M., Kaur, S., Gupta, A.K., Puranik, S., Singh, S., Singh, M., Gupta, S., Babu, B.K. & Sood, S. 2016a. Nutraceutical value of finger millet [Eleusine coracana (L.) Gaertn.], and their improvement using omics approaches. *Frontiers in Plant Science,* 7:934. https://doi.org/10.3389/fpls.2016.00934

Kumar, R., Kumar, A., Sharma, N.K., Kaur, N., Chunduri, V., Chawla, M., Sharma, S., Singh, K. & Garg, M. 2016b. Soft and hard textured wheat differ in starch properties as indicated by trimodal distribution, morphology, thermal and crystalline properties. *PloS One*, 11(1) e0147622. https://doi.org/10.1371/journal.pone.0147622

Kumar, V., Sinha, A.K., Makkar, H.P. & Becker, K. 2010. Dietary roles of phytate and phytase in human nutrition: A review. *Food Chemistry,* 120(4):945-959.

Kumari, M. & Platel, K. 2020. Impact of soaking, germination, fermentation, and thermal processing on the bioaccessibility of trace minerals from food grains. *Journal of Food Processing & Preservation,* 44(10):e14752. https://doi.org/10.1111/jfpp.14752

Kumari, P.L. & Sumathi, S. 2002. Effect of consumption of finger millet on hyperglycemia in non-insulin dependent diabetes mellitus (NIDDM) subjects. *Plant Foods for Human Nutrition,* 57(3-4):205-213.

Kunyanga, C.N., Imungi, J.K., Okoth, M.W., Biesalski, H.K. & Vadivel, V. 2012. Total phenolic content, antioxidant and antidiabetic properties of methanolic extract of raw and traditionally processed Kenyan indigenous food ingredients. *LWT-Food Science & Technology,* 45(2):269-276.

Łabanowska, M., Kurdziel, M., Bidzińska, E., Fortuna, T., Pietrzyk, S., Przetaczek-Rożnowska, I. & Rożnowski, J. 2013. Influence of metal ions on thermal generation of carbohydrate radicals in native and modified starch studied by EPR. *Starch-Stärke,* 65(5-6):469-482.

Lamy, E., Pinheiro, C., Rodrigues, L., Capela-Silva, F., Lopes, O., Tavares, S. & Gaspar, R. 2016. *Determinants of tannin-rich food and beverage consumption: oral perception vs. psychosocial aspects*. [Online] Available from: http://hdl.handle.net/10174/18018 [accessed: 15 April 2020].

Lau, S.K., Teng, J.L., Ho, C.-C. & Woo, P.C. 2015. Gene amplification and sequencing for bacterial identification. *Methods in Microbiology*, 42:433-464.

Le Bourvellec, C. & Renard, C. 2012. Interactions between polyphenols and macromolecules: quantification methods and mechanisms. *Critical Reviews in Food Science & Nutrition,* 52(3):213-248.

Léder, I. 2004. Sorghum and millets. *Cultivated Plants, Primarily as Food Sources,* 1:66-84.

Lee, S.H., Chung, I.-M., Cha, Y.-S. & Park, Y. 2010. Millet consumption decreased serum concentration of triglyceride and C-reactive protein but not oxidative status in hyperlipidemic rats. *Nutrition Research,* 30(4):290-296.

Lei, V. & Jakobsen, M. 2004. Microbiological characterization and probiotic potential of koko and koko sour water, African spontaneously fermented millet porridge and drink. *Journal of Applied Microbiology*, 96(2):384-397.

Lewis, J.K., Wei, J. & Siuzdak, G. 2006. Matrix-assisted laser desorption/ionization mass spectrometry in peptide and protein analysis. *Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation*. https://doi.org/10.1002/9780470027318.a1621

Li, P., Lin, W., Liu, X., Wang, X. & Luo, L. 2016. Environmental factors affecting microbiota dynamics during traditional solid-state fermentation of Chinese Daqu starter. *Frontiers in Microbiology*, 7:1237. https://doi.org/10.3389/fmicb.2016.01237

Li, Q., Chai, S., Li, Y., Huang, J., Luo, Y., Xiao, L. & Liu, Z. 2018. Biochemical components associated with microbial community shift during the pile-fermentation of primary dark tea. *Frontiers in Microbiology*, 9:1509. https://doi.org/10.3389/fmicb.2018.01509

Lin, J.-K., Chen, P.-C., Ho, C.-T. & Lin-Shiau, S.-Y. 2000. Inhibition of xanthine oxidase and suppression of intracellular reactive oxygen species in HL-60 cells by theaflavin-3, 3 '-digallate,(−)-epigallocatechin-3-gallate, and propyl gallate. *Journal of Agricultural & Food Chemistry*, 48(7):2736-2743.

Lindahl, M. & Tagesson, C. 1993. Selective inhibition of group II phospholipase A 2 by quercetin. *Inflammation*, 17(5):573-582.

Liu, P., Li, Y., Shang, X. & Xie, F. 2019. Starch–zinc complex and its reinforcement effect on starch-based materials. *Carbohydrate Polymers*, 206:528-538.

Liu, S., Ren, F., Zhao, L., Jiang, L., Hao, Y., Jin, J., Zhang, M., Guo, H., Lei, X. & Sun, E. 2015. Starch and starch hydrolysates are favorable carbon sources for Bifidobacteria in the human gut. *BMC Microbiology*, 15(1):1-9.

Lobo, V., Patil, A., Phatak, A. & Chandra, N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*, 4(8):118-126.

Lopez, H.W., Leenhardt, F., Coudray, C. & Remesy, C. 2002. Minerals and phytic acid interactions: is it a real problem for human nutrition? *International Journal of Food Science & Technology*, 37(7):727-739.

Lozupone, C. & Knight, R. 2005. UniFrac: a new phylogenetic method for comparing microbial communities. *Applied Environmental Microbiology*, 71(12):8228-8235.

Lozupone, C.A., Hamady, M., Kelley, S.T. & Knight, R. 2007. Quantitative and qualitative β diversity measures lead to different insights into factors that structure microbial communities. *Applied Environmental Microbiology*, 73(5):1576-1585.

Luo, Z., Zou, J., Chen, H., Cheng, W., Fu, X. & Xiao, Z. 2016. Synthesis and characterization of amylose–zinc inclusion complexes. *Carbohydrate Polymers*, 137:314-320.

Lv, X.-C., Jia, R.-B., Li, Y., Chen, F., Chen, Z.-C., Liu, B., Chen, S.-J., Rao, P.-F. & Ni, L. 2016. Characterization of the dominant bacterial communities of traditional fermentation starters for Hong Qu glutinous rice wine by means of MALDI-TOF mass spectrometry fingerprinting, 16S rRNA gene sequencing and species-specific PCRs. *Food Control*, 67:292-302.

Madoroba, E. 2011. *Molecular characterization and population dynamics of lactic acid bacteria during the fermentation of sorghum.* (Doctoral dissertation, University of Pretoria).

Makokha, A.O., Oniang'o, R.K., Njoroge, S.M. & Kamar, O.K. 2002. Effect of traditional fermentation and malting on phytic acid and mineral availability from sorghum (Sorghum bicolor) and finger millet (Eleusine coracana) grain varieties grown in Kenya. *Food & Nutrition Bulletin*, 23(3_suppl 1):241-245.

Mandal, S., Van Treuren, W., White, R.A., Eggesbø, M., Knight, R. & Peddada, S.D. 2015. Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microbial Ecology in Health & Disease*, 26(1):27663. http://dx.doi.org/10.3402/mehd.v26.27663

Marsh, A.J., Hill, C., Ross, R.P. & Cotter, P.D. 2014. Fermented beverages with health-promoting potential: past and future perspectives. *Trends in Food Science & Technology*, 38(2):113-124.

Marshall, V.M. 1987. Fermented milks and their future trends: I. Microbiological aspects. *Journal of Dairy Research*, 54(4):559-574.

Martinez-Villaluenga, C., Peñas, E. & Frias, J. 2017. Chapter 2 - Bioactive peptides in fermented foods: Production and evidence for health effects. *Fermented Foods in Health and Disease Prevention*, pp. 23-47. Academic Press, Boston.

Mayosi, B.M., Flisher, A.J., Lalloo, U.G., Sitas, F., Tollman, S.M. & Bradshaw, D. 2009. The burden of non-communicable diseases in South Africa. *The Lancet*, 374(9693):934-947.

Mccleary, B.V., Gibson, T.S. & Mugford, D.C. 1997. Measurement of total starch in cereal products by amyloglucosidase-α-amylase method: Collaborative study. *Journal of AOAC International*, 80(3):571-579.

McDonough, C., Rooney, L. & Earp, C. 1986. Structural characteristics of Eleusine corocana (finger millet) using scanning electron and fluorescence microscopy. *Food Structure*, 5(2):247-256.

McFeeters, R.F. 1988. Effects of fermentation on the nutritional properties of food. *Nutritional Evaluation of Food Processing*, 423-446. Springer, Dordrecht. https://doi.org/10.1007/978-94-011-7030-7_16

Mellen, P.B., Walsh, T.F. & Herrington, D.M. 2008. Whole grain intake and cardiovascular disease: a meta-analysis. *Nutrition, Metabolism & Cardiovascular Diseases*, 18(4):283-290.

Miguel, M.G.d.C.P., de Castro Reis, L.V., Efraim, P., Santos, C., Lima, N. & Schwan, R.F. 2017. Cocoa fermentation: Microbial identification by MALDI-TOF MS, and sensory evaluation of produced chocolate. *LWT-Food Science and Technology*, 77:362-369.

Milanović, V., Osimani, A., Garofalo, C., Belleggia, L., Maoloni, A., Cardinali, F., Mozzon, M., Foligni, R., Aquilanti, L. & Clementi, F. 2020. Selection of cereal-sourced lactic acid bacteria as candidate starters for the baking industry. *Plos One*, 15(7). https://doi.org/10.1371/journal.pone.0236190

Miller, D.D., Schricker, B.R., Rasmussen, R.R. & Van Campen, D. 1981. An in vitro method for estimation of iron availability from meals. *The American Journal of Clinical Nutrition*, 34(10):2248-2256.

Miller, N.J., Rice-Evans, C., Davies, M.J., Gopinathan, V. & Milner, A. 1993. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science*, 84(4):407-412.

Minekus, M. 1998. Development and validation of a dynamic model of the gastrointestinal tract [PhD thesis]. *Utrecht (The Netherlands): University Utrecht*.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carriere, F., Boutrou, R., Corredig, M. & Dupont, D. 2014. A standardised static in vitro digestion method suitable for food–an international consensus. *Food & Function,* 5(6):1113-1124.

Mohiedeen, I.E., Tinay, A., Elkhalya, A., Babiker, E.E. & Mallasiy, L. 2010. Effect of fermentation on in vitro protein digestibility, protein fractions and amino acids composition of maize (Zea mays Linnaus) cultivars. *Electronic Journal of Environmental & Agriculture Food Chemistry,* 9:838-847.

Mohite, B., Chaudhari, G., Ingale, H. & Mahajan, V. 2013. Effect of fermentation and processing on in vitro mineral estimation of selected fermented foods. *International Food Research Journal*, 20(3):1373-1377.

Moodley, S.S. 2015. *Investigating the microbiological profile of motoho, a fermented sorghum beverage.* (MSc Dissertation), University of Pretoria, Pretoria, South Africa.

Morales, G., Marquez, L., Hernández, A. & Moyano, F. 2016. Chapter 9 Phytase effects on protein and phosphorus bioavailability in fish diets. *Phytate destruction-consequences for precision animal nutrition*: Wageningen Academic Publishers.

Motarjemi, Y. & Nout, M. 1996. Food fermentation: a safety and nutritional assessment. Joint FAO/WHO Workshop on Assessment of Fermentation as a Household Technology for Improving Food Safety. *Bulletin of the World Health Organization,* 74(6):553.

Mugocha, P.T. 2001. *Fermentation of a finger millet-dairy composite gruel.* (PhD Thesis), University of Pretoria, Pretoria, South Africa.

Mugula, J., Nnko, S., Narvhus, J. & Sørhaug, T. 2003. Microbiological and fermentation characteristics of togwa, a Tanzanian fermented food. *International Journal of Food Microbiology,* 80(3):187-199.

Mutasa, M. & Ayebo, A. 1993. Fermentation of mahewu using a maize meal base. *Zimbabwe Science News,* 27(10-12):86-89.

Muthayya, S., Rah, J.H., Sugimoto, J.D., Roos, F.F., Kraemer, K. & Black, R.E. 2013. The global hidden hunger indices and maps: an advocacy tool for action. *PLoS One,* 8(6):e67860. https://doi.org/10.1371/journal.pone.0067860

Nair, B.M., Asp, N.-G., Nyman, M. & Persson, H. 1987. Binding of mineral elements by some dietary fibre components—in vitro (I). *Food Chemistry,* 23(4):295-303.

Nampoothiri, K.M., Beena, D.J., Vasanthakumari, D.S. and Ismail, B. 2017. Health benefits of exopolysaccharides in fermented foods. In *Fermented foods in health and disease prevention*. Academic Press,49-62.

Navarrete-Bolaños, J.L. 2012. Improving traditional fermented beverages: how to evolve from spontaneous to directed fermentation. *Engineering in Life Sciences*, *12*(4):410-418.

Nche, P., Nout, M. & Rombouts, F. 1995. The effects of processing on the availability of lysine in kenkey, a Ghanaian fermented maize food. *International Journal of Food Sciences & Nutrition,* 46(3):241-246.

Niessen, L.W., Mohan, D., Akuoku, J.K., Mirelman, A.J., Ahmed, S., Koehlmoos, T.P., Trujillo, A., Khan, J. & Peters, D.H. 2018. Tackling socioeconomic inequalities and non-communicable diseases in low-income and middle-income countries under the Sustainable Development agenda. *The Lancet,* 391(10134):2036-2046.

Nkhata, S.G., Ayua, E., Kamau, E.H. & Shingiro, J.B. 2018. Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food Science & Nutrition,* 6(8):2446-2458.

Nooshkam, M., Varidi, M. & Bashash, M. 2019. The Maillard reaction products as food-born antioxidant and antibrowning agents in model and real food systems. *Food Chemistry,* 275:644-660.

Norman, R., Bradshaw, D., Schneider, M., Joubert, J., Groenewald, P., Lewin, S., Steyn, K., Vos, T., Laubscher, R. & Nannan, N. 2007. A comparative risk assessment for South Africa in 2000: towards promoting health and preventing disease. *South African Medical Journal,* 97(8):637-641.

Nout, M. & Motarjemi, Y. 1997. Assessment of fermentation as a household technology for improving food safety: a joint FAO/WHO workshop. *Food Control,* 8(5-6):221-226.

Nout, M.R. 2009. Rich nutrition from the poorest–Cereal fermentations in Africa and Asia. *Food Microbiology,* 26(7):685-692.

Nyaaba, G.N., Stronks, K., Aikins, A.d.-G., Kengne, A.P. & Agyemang, C. 2017. Tracing Africa's progress towards implementing the Non-Communicable Diseases Global action plan 2013–2020: a synthesis of WHO country profile reports. *BMC Public Health,* 17(1):1-13.

Oates, C.G. 1997. Towards an understanding of starch granule structure and hydrolysis. *Trends in Food Science & Technology,* 8(11):375-382.

Oghbaei, M. & Prakash, J. 2016. Effect of primary processing of cereals and legumes on its nutritional quality: A comprehensive review. *Cogent Food & Agriculture,* 2(1):1136015. https://doi.org/10.1080/23311932.2015.1136015

Ogodo, A.C., Ugbogu, O.C., Onyeagba, R.A. & Okereke, H.C. 2019. Microbiological quality, proximate composition and in vitro starch/protein digestibility of Sorghum bicolor flour fermented with lactic acid bacteria consortia. *Chemical & Biological Technologies in Agriculture,* 6(1):1-9.

Olaniran, A., Abiose, S., Adeniran, H., Gbadamosi, S. & Iranloye, Y. 2020. Production of a cereal based product (Ogi): Influence of co-fermentation with powdered garlic and ginger on the microbiome. *Agrosearch,* 20(1):81-93.

Onyango, C., Noetzold, H., Ziems, A., Hofmann, T., Bley, T. & Henle, T. 2005. Digestibility and antinutrient properties of acidified and extruded maize–finger millet blend in the production of uji. *LWT-Food Science & Technology,* 38(7):697-707.

Orhun, G.E. 2013. Maize for life. *International Journal of Food Science & Nutrition Engineering,* 3(2):13-16.

Osman, M.A. 2011. Effect of traditional fermentation process on the nutrient and antinutrient contents of pearl millet during preparation of Lohoh. *Journal of the Saudi Society of Agricultural Sciences,* 10(1):1-6.

Owino, V.O. 2019. Challenges and opportunities to tackle the rising prevalence of diet-related non-communicable diseases in Africa. *Proceedings of the Nutrition Society,* 78(4):506-512.

Payling, L., Fraser, K., Loveday, S., Sims, I., Roy, N. & McNabb, W. 2020. The effects of carbohydrate structure on the composition and functionality of the human gut microbiota. *Trends in Food Science & Technology*, 97:233-248.

Phiri, S., Schoustra, S.E., van den Heuvel, J., Smid, E.J., Shindano, J. & Linnemann, A. 2019. Fermented cereal-based Munkoyo beverage: Processing practices, microbial diversity and aroma compounds. *PloS one*, 14(10):e0223501. https://doi.org/10.1371/journal.pone.0223501

Pinto, V.Z., Moomand, K., Vanier, N.L., Colussi, R., Villanova, F.A., Zavareze, E.R., Lim, L.T. & Dias, A.R. 2015. Molecular structure and granule morphology of native and heat-moisture-treated pinhão starch. *International Journal of Food Science & Technology*, 50(2):282-289.

Plaami, S. & Kumpulainen, J. 1995. Inositol phosphate content of some cereal-based foods. *Journal of Food Composition & Analysis*, 8(4):324-335.

Pranoto, Y., Anggrahini, S. & Efendi, Z. 2013. Effect of natural and Lactobacillus plantarum fermentation on in-vitro protein and starch digestibilities of sorghum flour. *Food Bioscience*, 2:46-52.

Prior, R.L., Wu, X. & Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural & Food Chemistry*, 53(10):4290-4302.

Qin, H., Sun, Q., Pan, X., Qiao, Z. & Yang, H. 2016. Microbial diversity and biochemical analysis of Suanzhou: a traditional Chinese fermented cereal gruel. *Frontiers in Microbiology*, 7:1311. https://doi.org/10.3389/fmicb.2016.01311

Raheem, D., Dayoub, M., Birech, R. & Nakiyemba, A. 2021. The contribution of cereal grains to food security and sustainability in Africa: Potential application of UAV in Ghana, Nigeria, Uganda, and Namibia. *Urban Science*, 5(1):8. https://doi.org/10.3390/urbansci5010008

Ramakrishnan, U. 2002. Prevalence of micronutrient malnutrition worldwide. *Nutrition Reviews*, 60(suppl_5):S46-S52.

Ramashia, S.E., Anyasi, T.A., Gwata, E.T., Meddows-Taylor, S. & Jideani, A.I.O. 2019. Processing, nutritional composition and health benefits of finger millet in sub-saharan Africa. *Food Science & Technology*, 39(2):253-266.

Ramos, C.L., de Almeida, E.G., Freire, A.L. & Schwan, R.F. 2011. Diversity of bacteria and yeast in the naturally fermented cotton seed and rice beverage produced by Brazilian Amerindians. *Food Microbiology,* 28(7):1380-1386.

Rathore, T., Singh, R., Kamble, D.B., Upadhyay, A. & Thangalakshmi, S. 2019. Review on finger millet: Processing and value addition. [Online] Available from: https://www.thepharmajournal.com/archives/2019/vol8issue4/PartE/8-4-16-516.pdf [accessed 29 October 2020].

Ratnayake, W. & Jackson, D. 2008. Chapter 5 Starch Gelatinization. *Advances in Food & Nutrition Research,* 55:221-268.

Ravindran, V., Cabahug, S., Ravindran, G. & Bryden, W. 1999. Influence of microbial phytase on apparent ileal amino acid digestibility of feedstuffs for broilers. *Poultry Science*, 78(5):699-706.

Ray, M., Ghosh, K., Singh, S. & Mondal, K.C. 2016. Folk to functional: an explorative overview of rice-based fermented foods and beverages in India. *Journal of Ethnic Foods,* 3(1):5-18.

Reale, A., Konietzny, U., Coppola, R., Sorrentino, E. & Greiner, R. 2007. The importance of lactic acid bacteria for phytate degradation during cereal dough fermentation. *Journal of Agricultural & Food Chemistry*, 55(8):2993-2997.

Reddy, N.R. & Sathe, S.K. 2001. *Food Phytates.* CRC Press, Boca Raton.

Reiner, K. 2010. Catalase test protocol. *American Society for Microbiology*, [Online] Available from: https://asm.org/getattachment/72a871fc-ba92-4128-a194-6f1bab5c3ab7/Catalase-Test-Protocol.pdf [accessed 04 October 2020].

Ren, X., Chen, J., Molla, M.M., Wang, C., Diao, X. & Shen, Q. 2016. In vitro starch digestibility and in vivo glycemic response of foxtail millet and its products. *Food & Function,* 7(1):372-379.

Resende, L.V., Pinheiro, L.K., Miguel, M.G.d.C.P., Ramos, C.L., Vilela, D.M. & Schwan, R.F. 2018. Microbial community and physicochemical dynamics during the production of 'Chicha', a traditional beverage of Indigenous people of Brazil. *World Journal of Microbiology & Biotechnology*, 34(3):1-11.

Rousseau, S., Kyomugasho, C., Celus, M., Hendrickx, M.E. & Grauwet, T. 2020. Barriers impairing mineral bioaccessibility and bioavailability in plant-based foods and the perspectives for food processing. *Critical Reviews in Food Science & Nutrition,* 60(5):826-843.

Sagum, R. & Arcot, J. 2000. Effect of domestic processing methods on the starch, non-starch polysaccharides and in vitro starch and protein digestibility of three varieties of rice with varying levels of amylose. *Food Chemistry,* 70(1):107-111.

Salar, R.K., Certik, M. & Brezova, V. 2012. Modulation of phenolic content and antioxidant activity of maize by solid state fermentation with Thamnidium elegans CCF 1456. *Biotechnology & Bioprocess Engineering,* 17(1):109-116.

Saleh, A.S., Zhang, Q., Chen, J. & Shen, Q. 2013. Millet grains: nutritional quality, processing, and potential health benefits. *Comprehensive Reviews in Food Science & Food Safety,* 12(3):281-295.

San Miguel-Chávez, R. 2017. Phenolic antioxidant capacity: A review of the state of the art. *Phenolic Compounds - Biological Activity,* [Online] Available from: https://www.intechopen.com/books/phenolic-compounds-biological-activity/phenolic-antioxidant-capacity-a-review-of-the-state-of-the-artSan [accessed 15 January 2021].

Sandberg, A.-S. 2005. Methods and options for in vitro dialyzability; benefits and limitations. *International Journal for Vitamin & Nutrition Research,* 75(6):395-404.

Satish Kumar, R., Kanmani, P., Yuvaraj, N., Paari, K., Pattukumar, V. & Arul, V. 2013. Traditional Indian fermented foods: a rich source of lactic acid bacteria. *International Journal of Food Sciences & Nutrition,* 64(4):415-428.

Schönfeldt, H.C. & Hall, N.G. 2012. Dietary protein quality and malnutrition in Africa. *British Journal of Nutrition,* 108(S2):S69-S76.

Schutte, A.E. 2019. Urgency for South Africa to prioritise cardiovascular disease management. *The Lancet Global Health,* 7(2):e177-e178.

Selle, P., Ravindran, V., Caldwell, A. & Bryden, W. 2000. Phytate and phytase: consequences for protein utilisation. *Nutrition Research Reviews,* 13(2):255-278.

Serhan, C.N. & Savill, J. 2005. Resolution of inflammation: the beginning programs the end. *Nature Immunology,* 6(12):1191-1197.

Shahidi, F. & Chandrasekara, A. 2013. Millet grain phenolics and their role in disease risk reduction and health promotion: A review. *Journal of Functional Foods,* 5(2):570-581.

Sharma, D., Jamra, G., Singh, U.M., Sood, S. & Kumar, A. 2017. Calcium biofortification: three pronged molecular approaches for dissecting complex trait of calcium nutrition in finger millet (Eleusine coracana) for devising strategies of enrichment of food crops. *Frontiers in Plant Science,* 7:2028. https://doi.org/10.3389/fpls.2016.02028

Shi, J., Shan, S., Li, H., Song, G. & Li, Z. 2017. Anti-inflammatory effects of millet bran derived-bound polyphenols in LPS-induced HT-29 cell via ROS/miR-149/Akt/NF-κB signaling pathway. *Oncotarget,* 8(43):74582-74594.

Shobana, S., Krishnaswamy, K., Sudha, V., Malleshi, N., Anjana, R., Palaniappan, L. & Mohan, V. 2013. Finger millet (Ragi, Eleusine coracana L.): a review of its nutritional properties, processing, and plausible health benefits. *Advances in Food & Nutrition Research,* 69:1-39.

Shobana, S., Sreerama, Y. & Malleshi, N. 2009. Composition and enzyme inhibitory properties of finger millet (Eleusine coracana L.) seed coat phenolics: Mode of inhibition of α-glucosidase and pancreatic amylase. *Food Chemistry,* 115(4):1268-1273.

Shumoy, H., Lauwens, S., Gabaza, M., Vandevelde, J., Vanhaecke, F. & Raes, K. 2017. Traditional fermentation of tef injera: Impact on in vitro iron and zinc dialysability. *Food Research International,* 102:93-100.

Sigdel, A. 2019. Effect of Mineral Ions on the Functional Properties of Starch Films. [Online] Available from: https://openprairie.sdstate.edu/etd/3256 [accessed 24 April 2021].

Simango, C. 1997. Potential use of traditional fermented foods for weaning in Zimbabwe. *Social Science & Medicine,* 44(7):1065-1068.

Simango, C. & Rukure, G. 1992. Survival of bacterial enteric pathogens in traditional fermented foods. *Journal of Applied Bacteriology,* 73(1):37-40.

Simango, C. & Rukure, G. 1991. Survival of Campylobacter jejuni and pathogenic Escherichia coli in mahewu, a fermented cereal gruel. *Transactions of the Royal Society of Tropical Medicine & Hygiene,* 85(3):399-400.

Singh, M. & Kimothi, G. 2018. Phytochemical estimation and antioxidative potential of thunbergia mysorensis (wight) t. Anders. Ex bedd in uttarakhand, India. *International Journal of Pharma & Bio Sciences*, 9(3):271-279.

Singh, N., Kaur, A. & Shevkani, K. 2014. Maize: grain structure, composition, milling, and starch characteristics. In: Chaudhary, D., Kumar, S. & Langyan, S. (Eds.), *Maize: Nutrition Dynamics and Novel Uses*, pp. 65-76. Springer, New Delhi. https://doi.org/10.1007/978-81-322-1623-0_5

Singh, N., Singh, J., Kaur, L., Sodhi, N.S. & Gill, B.S. 2003. Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chemistry*, 81(2):219-231.

Singhal, N., Kumar, M., Kanaujia, P.K. & Virdi, J.S. 2015. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. *Frontiers in Microbiology*, 6:791. http://dx.doi.org/10.3389/fmicb.2015.00791

Singleton, V.L., Orthofer, R. & Lamuela-Raventós, R.M. 1999. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, 299:152-178.

Siwela, M. 2009. *Finger millet grain phenolics and their impact on malt and cookie quality.* (PhD Thesis), University of Pretoria, Pretoria, South Africa.

Siwela, M., Taylor, J.R., de Milliano, W.A. & Duodu, K.G. 2010. Influence of phenolics in finger millet on grain and malt fungal load, and malt quality. *Food Chemistry*, 121(2):443-449.

Sofi, P., Wani, S.A., Rather, A. & Wani, S.H. 2009. Quality protein maize (QPM): Genetic manipulation for the nutritional fortification of maize. *Journal of Plant Breeding & Crop Science*, 1(6):244-253.

Songré-Ouattara, L., Mouquet-Rivier, C., Icard-Vernière, C., Humblot, C., Diawara, B. & Guyot, J.-P. 2008. Enzyme activities of lactic acid bacteria from a pearl millet fermented gruel (ben-saalga) of functional interest in nutrition. *International Journal of Food Microbiology*, 128(2):395-400.

Spires, M., Delobelle, P., Sanders, D., Puoane, T. & Swart, R. 2016. Diet related non-communicable diseases in South Africa: determinants and policy response. *South Africa Health Reviews*,1: 35-42.

Sripriya, G., Antony, U. & Chandra, T. 1997. Changes in carbohydrate, free amino acids, organic acids, phytate and HCl extractability of minerals during germination and fermentation of finger millet (Eleusine coracana). *Food Chemistry*, 58(4):345-350.

Steinkraus, K.H. 1997. Classification of fermented foods: worldwide review of household fermentation techniques. *Food Control*, 8(5-6):311-317.

Stoltzfus, R.J. 2001. Defining iron-deficiency anemia in public health terms: a time for reflection. *The Journal of Nutrition*, 131(2):565S-567S.

Stoltzfus, R.J. 2003. Iron deficiency: global prevalence and consequences. *Food & Nutrition Bulletin*, 24(4_suppl_1):S99-S103.

Sujka, M. & Jamroz, J. 2007. Starch granule porosity and its changes by means of amylolysis. *International Agrophysics*, 21(1):107-113.

Svanberg, U., Lorri, W. & Sandbeag, A.S. 1993. Lactic fermentation of non-tannin and high-tannin cereals: effects on in vitro estimation of iron availability and phytate hydrolysis. *Journal of Food Science*, 58(2):408-412.

Svihus, B., Uhlen, A.K. & Harstad, O.M. 2005. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. *Animal Feed Science & Technology*, 122(3-4):303-320.

Taylor, J. & Taylor, J.R.N. 2002. Alleviation of the adverse effect of cooking on sorghum protein digestibility through fermentation in traditional African porridges. *International Journal of Food Science & Technology*, 37(2):129-137.

Taylor, J.R.N. 2017. Millets: Their unique nutritional and health-promoting attributes. In: Taylor J.R.N. & Awika, J.M. (Eds.), *Gluten-Free Ancient Grains*: Woodhead Publishing, pp. 55-103.

Taylor, J.R.N., Belton, P.S., Beta, T. & Duodu, K.G. 2014. Increasing the utilisation of sorghum, millets and pseudocereals: Developments in the science of their phenolic

phytochemicals, biofortification and protein functionality. *Journal of Cereal Science,* 59(3):257-275.

Taylor, J.R.N. & Duodu, K.G. 2015. Effects of processing sorghum and millets on their phenolic phytochemicals and the implications of this to the health-enhancing properties of sorghum and millet food and beverage products. *Journal of the Science of Food & Agriculture,* 95(2):225-237.

Taylor, J.R.N. & Emmambux, M.N. 2008. Gluten-free foods and beverages from millets. In: Arendt, E.K. & Dal Bello, F. (Eds.), *Gluten-Free Cereal Products and Beverages*: Academic Press, pp. 119-148.

Temba, M.C., Njobeh, P.B., Adebo, O.A., Olugbile, A.O. & Kayitesi, E. 2016. The role of compositing cereals with legumes to alleviate protein energy malnutrition in Africa. *International Journal of Food Science & Technology,* 51(3):543-554.

Teniola, O. & Odunfa, S. 2001. The effects of processing methods on the levels of lysine, methionine and the general acceptability of ogi processed using starter cultures. *International Journal of Food Microbiology,* 63(1-2):1-9.

Tharanathan, R. 1995. Starch: The polysaccharide of high abundance and usefulness. *Journal of Scientific & Industrial Research,* 54(8):452-458.

Tharanathan, R., Muralikrishna, G., Salimath, P. & Rao, M.R. 1987. Plant carbohydrates—an overview. *Proceedings: Plant Sciences,* 97(2):81-155.

Thompson, B. 2007. Food-based approaches for combating iron deficiency. *Nutritional Anemia,* 337:1-21.

Tontisirin, K., Nantel, G. & Bhattacharjee, L. 2002. Food-based strategies to meet the challenges of micronutrient malnutrition in the developing world. *Proceedings of the Nutrition Society,* 61(2):243-250.

Towo, E., Matuschek, E. & Svanberg, U. 2006. Fermentation and enzyme treatment of tannin sorghum gruels: effects on phenolic compounds, phytate and in vitro accessible iron. *Food Chemistry,* 94(3):369-376.

Vaidya, R.H. & Sheth, M.K. 2011. Processing and storage of Indian cereal and cereal products alters its resistant starch content. *Journal of Food Science & Technology,* 48(5):622-627.

Valko, M., Izakovic, M., Mazur, M., Rhodes, C.J. & Telser, J. 2004. Role of oxygen radicals in DNA damage and cancer incidence. *Molecular & Cellular Biochemistry,* 266(1-2):37-56.

Van der Merwe, R. 2017. *Pearl millet porridge: Improvement in iron and zinc bioaccessibilities through fortification with micronutrient-rich plant food components.* (MSc Dissertation), University of Pretoria, Pretoria, South Africa.

Verma, V.C., Kumar, A., Zaidi, M., Verma, A., Jaiswal, J., Singh, D., Singh, A. & Agrawal, S. 2018. Starch isolation from different cereals with variable amylose/amylopectin ratio and its morphological study using SEM and FT-IR. *International Journal of Current Microbiology & Applied Sciences,* 7(10):211-228.

Vilela, A. 2019. The importance of yeasts on fermentation quality and human health-promoting compounds. *Fermentation,* 5(2):46. http://dx.doi.org/10.3390/fermentation5020046

Walker, C.F., Ezzati, M. & Black, R. 2009. Global and regional child mortality and burden of disease attributable to zinc deficiency. *European Journal of Clinical Nutrition,* 63(5):591-597.

Walter, M. & Marchesan, E. 2011. Phenolic compounds and antioxidant activity of rice. *Brazilian Archives of Biology & Technology,* 54(2):371-377.

Wang, X., Jordan, I.K. & Mayer, L.W. 2015. A phylogenetic perspective on molecular epidemiology. In: Tang, Y., Sussman, M., Liu, D., Poxton, I. & Schwartzman, J. (Eds.), *Molecular Medical Microbiology*: Academic Press, pp. 517-536.

Watanabe, M. 1999. Antioxidative phenolic compounds from Japanese barnyard millet (Echinochloa utilis) grains. *Journal of Agricultural & Food Chemistry,* 47(11):4500-4505.

Wessells, K.R. & Brown, K.H. 2012. Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. *PloS One,* 7(11):e50568.

WHO 2015. The global prevalence of anaemia in 2011. Geneva, Switzerland: WHO Press. [Online] Available from: http://apps.who.int/iris/bitstream/10665/177094/1/9789241564960_eng.pdf?ua=1&ua=1 [accessed 29 October 2020].

WHO/UNICEF/UNU. 2001. *Iron deficiency anaemia assessment, prevention and control: a guide for programme managers.*: Geneva, Switzerland: WHO Press.

Wisotzkey, J.D., Jurtshuk JR, P., Fox, G.E., Deinhard, G. & Poralla, K. 1992. Comparative sequence analyses on the 16S rRNA (rDNA) of Bacillus acidocaldarius, Bacillus acidoterrestris, and Bacillus cycloheptanicus and proposal for creation of a new genus, Alicyclobacillus gen. nov. *International Journal of Systematic & Evolutionary Microbiology,* 42(2):263-269.

Wolters, M.G., Schreuder, H.A., Van Den Heuvel, G., Van Lonkhuijsen, H.J., Hermus, R.J. & Voragen, A.G. 1993. A continuous in vitro method for estimation of the bioavailability of minerals and trace elements in foods: application to breads varying in phytic acid content. *British Journal of Nutrition,* 69(3):849-861.

Xu, Q.-S., Yan, Y.-S. & Feng, J.-X. 2016. Efficient hydrolysis of raw starch and ethanol fermentation: a novel raw starch-digesting glucoamylase from Penicillium oxalicum. *Biotechnology for Biofuels,* 9(1):1-18.

Yan, H.-B., Pan, X.-X., Jiang, H.-W. & Wu, G.-J. 2009. Comparison of the starch synthesis genes between maize and rice: copies, chromosome location and expression divergence. *Theoretical & Applied Genetics,* 119(5):815-825.

Yun, J.-S., Wee, Y.-J. & Ryu, H.-W. 2003. Production of optically pure L (+)-lactic acid from various carbohydrates by batch fermentation of Enterococcus faecalis RKY1. *Enzyme & Microbial Technology,* 33(4):416-423.

Zhang, L.Z. & Liu, R.H. 2015. Phenolic and carotenoid profiles and antiproliferative activity of foxtail millet. *Food Chemistry,* 174:495-501.

Zhang, Z., Blake, D.R., Stevens, C.R., Kanuler, J.M., Ward, P.G., Symons, M.C., Benboubetra, M. & Harrison, R. 1998. A reappraisal of xanthine dehydrogenase and oxidase in hypoxic reperfusion injury: the role of NADH as an electron donor. *Free Radical Research,* 28(2):151-164.

Zhao, H.-M., Guo, X.-N. & Zhu, K.-X. 2017. Impact of solid state fermentation on nutritional, physical and flavor properties of wheat bran. *Food Chemistry,* 217:28-36.

Zhu, F. 2015. Interactions between starch and phenolic compound. *Trends in Food Science & Technology,* 43(2):129-143.

Zvauya, R., Mygochi, T. & Parawira, W. 1997. Microbial and biochemical changes occurring during production of masvusvu and mangisi, traditional Zimbabwean beverages. *Plant Foods for Human Nutrition*, 51(1):43-51.